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THE WATER RELATIONS OF SNAILS AND SLUGS

III. FACTORS DETERMINING ACTIVITY IN *HELIX POMATIA* L.

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(With Four Text-figures)

I. INTRODUCTION

This paper leaves out of account the special case of hibernation, and deals with the activity phases of snails at other times.

It has long been known that both the water content and the activity of *Helix pomatia* vary very greatly, and there appears to be a broad parallelism between the two. Aestivating snails have a lower total water content, and a lower osmotic pressure of the blood, than active (Duval, 1930; Arvanitaki & Cardot, 1932; Meyer & Thibaudet, 1937). Even among non-aestivating animals, the correlation is said to apply; thus Duval (1930) writes of the blood of snails collected in the field, 'on voit très nettement, on observant les animaux avant de prélever leur milieu intérieur, que plus la concentration de ce milieu est grande, moins l'animal est actif. C'est ainsi qu'un individu donc le sang se congelait a −0·39° était presque inerte, à moitié rentré dans sa coquille. Ceux que l'on rencontre au contraire se promenant dans les herbes mouillées par la rosée du matin ont un sang se congelant aux environs de −0·30°.' We may infer that water content determines activity, or that activity determines water content, or that both are determined by other factors.

Does water content determine activity?

The view that activity depends on hydration was championed especially by Duval (1930), who describes as follows the alternation of aestivation and activity: 'L'animal étant collé, inerte, le Δ de son sang est inférieur à −0·40° et s'abaisse peu à peu. Une cause hydratante intervient-elle, pluie, rosée etc., la concentration du sang s'abaisse et quand elle devient inférieure à celle correspondant à Δ = −0·40°, l'animal s'éveille, sort de sa coquille, rampe, cherche sa nourriture, s'alimente....Lorsque la cause d'hydratation a cessé, l'animal se déshydrate assez rapidement et quand le Δ de son sang atteint −0·40° il redevient inerte, rentré dans sa coquille. Il restera dans cet état d'engourdissement jusqu'à ce qu'une cause d'hydratation vienne de nouveau faire remonter le Δ. Si cette cause ne se produit pas, l'animal se déshydrate progressivement et finit par mourir sans être redevenu actif.'

If this account be true, it should be possible, by experimentally varying the snail's water content, to control its activity. Attempts to do so, using *H. pomatia* and *H. aspersa*, were described in a doctorate thesis on 'la vie ralentie' by Fischer (1931). His conclusions are briefly summarized, without further experimental substantiation, in a preliminary note by Fischer & Duval (1931). This note has been widely quoted in favour of the view that water content may determine activity, e.g. by Pieh (1936) and by Schlieper (1936); the more extensive thesis, on which the note is based, is less well known. The present writer does not believe that Fischer's actual experimental results justify his conclusions; the point is of sufficient importance to deserve full discussion, particularly as Fischer's thesis is somewhat inaccessible.

Fischer's experiments on 'l'influence de la teneur en eau' on respiratory rate were inspired by a paper by Mayer & Plantefol (1925), who found that the oxygen consumption of mosses increased with hydration up to a maximum at a water content of 62–64%, above which it decreased again. He concluded from his results that the same general law held for snails, the maximum respiration occurring in this case at a total water content of 85–86%, and wrote: 'Il était intéressant d'étendre à un organisme animal les faits établis pour les mousses, et dont la portée générale avait été pressentie par les auteurs.'

This conclusion rests upon four propositions, in which he summarizes his actual findings. The fourth of these, about the respiratory quotient, does not now concern us; the others are as follows:

'1. *A une hydratation de* 80%, *la respiration est moins active qu'à une hydratation de* 85–90%.

'2. *Si chez un Escargot peu riche en eau* (80% *par exemple*), *il se produit une déshydratation, celle-ci est accompagnée d'une diminution de l'intensité respiratoire; l'hydratation produit un résultat inverse.*

'3. *Une hydratation dépassant* 88% *est accompagnée au contraire d'une diminution de l'intensité respiratoire.*'

If, now, we turn to the descriptions of the actual experiments, we find that the evidence for the view that water content determines oxygen consumption is in reality exceedingly tenuous. The three propositions will now be examined one by one from this standpoint.

Proposition 1. This statement is based on a comparison between the oxygen consumption of hibernating snails (water content about 80%) with that of active ones (85–90%). In other words, the two groups of animals were not strictly comparable. It is well known that hibernating snails can be awakened by immersion in water, but, as Fischer himself points out in another chapter, the same thing can be done by exposure to light, warmth, vibration or low oxygen tension; 'l'humidité n'est pas un facteur nécessaire du réveil.' He also repeats and confirms an experiment by Schuurmans-Stekhoven (1920), who found that mere removal of the epiphragm of a hibernating snail nearly doubled the respiratory rate. Hibernation seems to resemble sleep, from which one can be aroused by divers stimuli. As the first thing the snail does, when it emerges, is to seek water and drink it, the higher activity of non-hibernating specimens may be the cause, rather than the result, of their raised water content.

Proposition 2. The first clause is based on the records of three hibernating snails, which slowly lost weight, mainly by evaporation of water, over a period of several weeks. In one of the three, the oxygen consumption steadily fell at the same time (three determinations were made). In the other two, whose water loss was assisted by P_2O_5, the respiratory rate fell on the whole, but with notable fluctuations, so its dependence on water content was apparently not at all strict. The second clause is based on a single experiment in which a hibernating snail, put into water, absorbed water and became active; the same criticism applies to this case as to proposition 1.

Proposition 3. This is supported by the records of three active animals. One hydrated itself spontaneously to a total water content of 90·05%; the others were artificially hydrated by immersion in water. The difficulty here is that the depression of respiration does not coincide very well in time with the increase of water content: 'La diminution de la consommation d'oxygène peut se produire avec un certain retard, et persiste quelque temps même si l'hydratation cesse.' Evidently, if there is a relationship between the two variables, it is by no means a simple and straightforward one.

The short note by Fischer & Duval (1931) adds nothing to the above; it simply states Fischer's general conclusions, and cites a portion of one of his protocols. It will, I think, be clear from the above account that the experiments of these workers have failed to provide convincing proof that hydration directly influences oxygen consumption in snails.

Does activity determine water content?

A very different picture of the relationship between water content and activity was given by Arvanitaki & Cardot (1932), in a paper on the variations in blood concentration of four *Helix* species. They showed that hydration depends in a rather indirect way on the atmospheric moisture, and very directly on the availability of food. Two experiments of great importance in the present context will be cited.

In the first experiment, a number of active *H. vermiculata* were collected from a garden at Tamaris, 18 hr. after a strong nocturnal rain, and the concentration of their blood was determined by measuring the conductivity. Another group, which had been kept for 15 days in the same garden, but confined in a cage ('nasse'), was similarly treated at the same time. Both groups had experienced identical atmospheric conditions, including exposure to rain. The only difference was that those in the cage had been unable to reach food. The blood of the fasting animals proved to be as concentrated as it is 'au cours des périodes d'estivation sévère', while that of the others was nearly twice as dilute.

In the second experiment, a great number of *H. pisana* were collected from the same garden as they fed after a heavy rain, which followed on a prolonged period of drought. They were then kept in dry vessels in the laboratory. They slowly lost weight from the moment of collection, a loss to which evaporation of water contributed, but their blood (which was very concentrated at the time of collection, although the animals had been active for some hours) became more and more dilute for 2 days, after which it rose quite rapidly to the usual aestivating concentration; at the same time, the cessation of copious defaecation showed that digestion was completed.

Arvanitaki & Cardot conclude from these observations that 'la dilution de l'hémolymphe après la pluie débute avec la digestion des aliments ingérés et cesse dès que celle-ci prend fin. Les fortes concentrations humorales de l'hibernation et de l'estivation sont la conséquence immédiate et directe du jeûne.' From these results, it appears that rain, after drought, does not so much hydrate an aestivating snail as stimulate it to emerge, eat and drink; that tissue hydration follows after a lag of over 12 hr. due to the slowness of absorption from the gut; and that the hydration is only measurable if the animal has fed.

Spontaneous variations in activity

In the views discussed above, the pace-maker controlling snail activity is environmental moisture, acting either as a stimulus to feed or by hydrating the tissues. But activity may fluctuate very widely in a manner apparently independent of any environmental variable. Howes & Wells (1934*a*) found that, if *H. pomatia* are kept under reasonably favourable conditions they undergo enormous oscillations in weight, the graphs presenting an irregularly cyclical character with a period of several days. The weight rises are due partly to feeding and partly to drinking. In the absence of food, similar fluctuations, but of reduced amplitude, appear. Activity varies with weight, for the snails creep about when their weight is high, and often aestivate, or at least withdraw into their shells, when it is low. Animals kept together in the same small container often show weight peaks on different days; the fluctuations are therefore of spontaneous, and not environmental, origin. The fluctuations were shown by every one of 114 snails studied, and even appeared, although with reduced amplitude, in some kept in atmospheres saturated with water vapour; Howes & Wells concluded: 'We have come to believe that a constant water content never occurs in the active phases of the life of this species.' Activity appears, then, to be determined, partly by environmental factors, especially moisture, and partly by an internal rhythm.

Similar cycles, of even greater amplitude, occur in newly hatched *H. pomatia* kept at 18–20° in atmospheres saturated, or nearly saturated, with water vapour (Howes & Whellock, 1937).

The experiments described below were made from time to time since the completion of Howes & Wells's paper, to throw further light on the nature of the cycles. After their detailed description, the discussion of activity in relation to internal and external variables will be resumed.

II. WEIGHT CYCLES UNDER CONSTANT CONDITIONS

Howes & Wells (1934*a*) regarded the weight cycles as due primarily to factors intrinsic to the snail, and not as responses to varying environmental conditions, because different animals kept in the same container showed peaks on different days. To confirm this conclusion, I kept a number of *H. pomatia* under constant conditions (of humidity, temperature, illumination, food supply and water supply) and weighed them daily.

The apparatus was originally designed by Stoughton (1930, 1931) and set up at Rothamsted for studying plant growth. It was later moved to University College, London, and used for the snail experiments. Only the upper half of Stoughton's apparatus was used. It consists essentially of a large box, with walls and roof of two layers of glass (enclosing an insulating air space) in wooden frames. The air inside is warmed by a coil of resistance wire, controlled by an electric thermoregulator and relay. A current of air is continually driven into the box; water evaporates into this air current from a piece of wet muslin lying on an electrically heated plate; the heater is controlled, through a relay, by a hair hygrometer inside the box. A combined recording thermometer and hygrometer, standing in the box, provides a check on the regulating devices. The snails were kept in the box, in containers of glass and perforated zinc (Howes & Wells, 1934*b*, Fig. 1). They were taken out and weighed daily.

The snails were received from France, in the hibernating condition, on 13 March 1934, and kept in a moderately cool refrigerator. When needed, they were 'brought out' by removal of the epiphragm and exposure to warmth and light in a shallow layer of water. Immediately after removal of the epiphragm, the hibernating weight was determined, and each snail was thereafter distinguished by a number painted on its shell. Following the routine of Howes & Wells (1934*a*), all subsequent weighings were converted into percentages of the hibernating weight of the animal concerned; this means that measurements made on different individuals are roughly comparable.

Two experiments were made. In the first, the animals were 'brought out' on 4 April 1934, and studied in the box from 24 April to 8 June. Some of these snails were given food (mixed carrot and cabbage) and a vessel of drinking water—both renewed daily—until 23 May, after which date they received water only. Others received water, but no food, from the time when they were 'brought out'. It is a remarkable fact that, of the latter group, two (out of nine) survived the whole experiment, although they had taken no nourishment since they began to hibernate. This experiment was run at 16° C. and 90–93% R.H. (except that, on 25 April, owing to a failure of the relay, the R.H. fell to 86%). At first the animals were continually illuminated, but, after 3 May, they were kept in darkness, except, of course, for the daily weighing.

In the second experiment, another set of animals from the same hibernating stock was 'brought out on 20 September 1934, and at once put in the box, some receiving carrot, cabbage and water and the others water only. The experiment was run until 14 October at 23° C. and 89–92% R.H. (except that the relay failed again on 3 October, and the R.H. fell to 82%). The snails were in darkness.

Two typical extracts from the records are shown in Fig. 1. The white and black circles give a rough idea of the activity of the snails when taken for their daily weighings. Actually, a number of grades of activity were noted on the protocols. The snail might be feeding or creeping along; or sitting still with most of its body out of the shell but its head more or less completely withdrawn; or sitting on the hinder half of the foot only, the rest being withdrawn; or withdrawn into the shell except that the tip of the heel was still visible, though not in contact with the substratum; or completely withdrawn, so that only the mantle could be seen in the mouth of the shell. In the latter case, the opening of the shell might or might not be closed with a film of dried mucus. For the purposes of the diagram, this series of states has been arbitrarily divided into 'active' (white circles) and 'resting' (black); the former group includes all states in which any part of the foot is applied to the substratum.

In all essentials, the records resemble those already published by Howes & Wells (1934*a*). The fed snails show enormous, irregularly cyclical variations in weight, the fasting ones show similar cycles of lesser amplitude, and both tend to be active on the weight peaks and withdrawn in the troughs. As before, there is no correlation, in either experiment, in the days on which different individuals peak. The conclusion that the cycles are not determined by varying environmental factors is therefore fully confirmed.

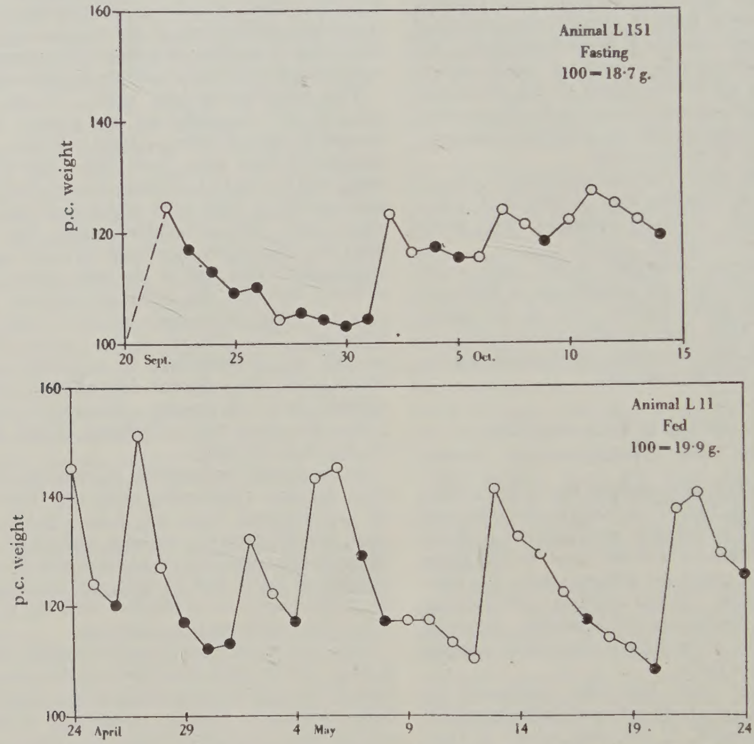

Fig. 1. Weight cycles in two snails kept under constant conditions. The upper record is of a fasting, the lower of a fed, snail. At the white circles the animals were 'active', at the black they were 'resting'.

III. THE RELATIONSHIP BETWEEN WEIGHT AND ACTIVITY

The results of Howes & Wells (1934*a*), and those described in the last section, show that there is some sort of relationship between weight and activity, for the snails tend to withdraw into their shells in the troughs of the weight curve. To get more precise information on this relationship, a number of oxygen consumption measurements were made on *H. pomatia*, at different stages of the weight cycle.

The measurements were made with a manometric apparatus already described (Wells, 1938). The general method of a single measurement was as follows. A snail was weighed, then put in the apparatus, where it found itself in a fairly spacious cylindrical glass container, at a constant temperature which varied in different experiments from 22 to 24° C. The air was exposed to 20% KOH, which absorbed CO_2 and, incidentally, regulated the humidity at about 85% R.H. After an hour and a half, during which time temperature equilibrium was reached and the snail could get used to its surroundings, the taps were closed and the oxygen consumption was followed manometrically for one hour. The snail was then returned to its vivarium.

The whole of the data are plotted in Fig. 2. Each point is a single measurement, made as just described. Weight is given as percentage of the hibernating weight. Oxygen consumption is given as c.c. (N.T.P.) per kilogram of hibernating weight per hour.

The details of the various experiments are as follows:

(1) A single preliminary measurement was made on each of fourteen snails. These were received from France in the hibernating state on 13 March 1934, and kept in the refrigerator until 20 September, when they were 'brought out', after determination of their hibernating weights, with warmth, water,

light and food. The respiration tests were made on 6–13 December, after they had been active for over 2 months. The results are shown as large white circles in Fig. 2.

(2) A stock of snails was received, hibernating, on 17 December 1934, and put in the refrigerator. On 29 March 1935 they were moved into the laboratory and divided into two groups, one (27 animals) receiving food and water and the other (13 animals) water only. Three measurements were made on each snail—the first soon after emergence from hibernation (on 31 March to 2 April), the second after about five days (5–7 April) and the third a week later (11–14 April). The resulting points are shown as small circles on Fig. 2; the consecutive measurements on any one snail are joined by straight lines; the fed animals are shown as white circles while those which had had no food since they began to hibernate are black.

(3) After the experiment just described, all the animals were kept with food and drink until 21 July, when two groups were selected. The first group, of seven snails, continued to receive food and water; the second, of five, got water only. Measurements of each of these animals were then made every day for 10 days. The results of the first 2 days were later discarded, in the case of the fasting snails, as representing a transition between the fed and the fasting conditions. All these measurements, except the discards just mentioned, are shown on Fig. 2 as small circles not joined by lines; as before, the fasting animals are black. The histories of six typical animals, from this experiment, are also plotted in Fig. 3.

(4) Another stock was received on 27 March 1935, and kept in the refrigerator until 7 December, when they were 'brought out' and fed. Measurements of these snails were made by Mr A. N. Rowan, using the author's apparatus, at various times from January to June of the following year. His points appear as medium-sized circles in Fig. 2; they include in all seventy-seven measurements, on forty-one animals, each of which was used from one to four times.

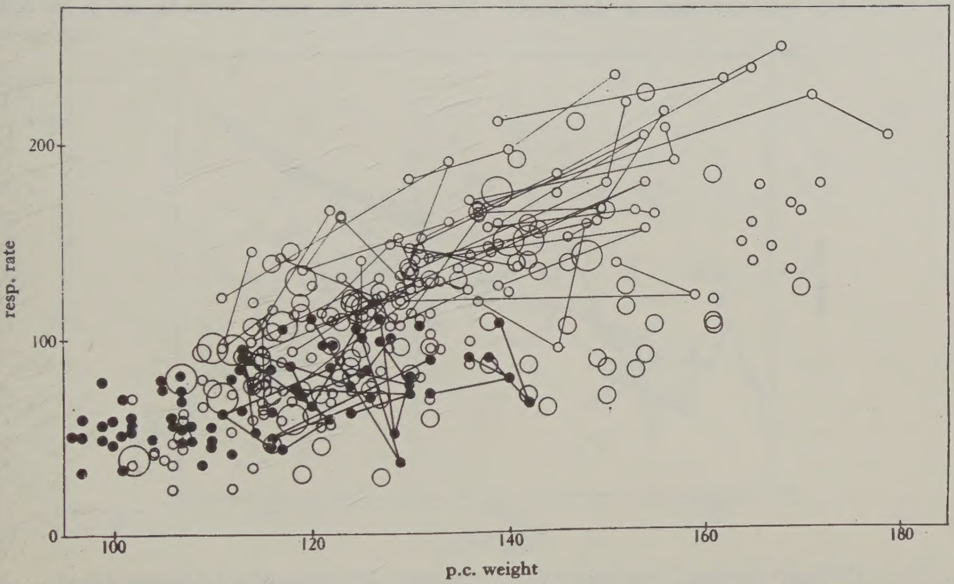

Fig. 2. Relationship between weight and oxygen consumption. White circles are fed animals, the three sizes denoting three stocks used in different years. Black circles are fasting animals. Straight lines join points obtained from the same snail.

When the data are considered, the following results emerge:

(1) In Fig. 2, the points are scattered over a broad diagonal belt of the diagram, so it seems that weight and activity are in fact related. The area covered is about the same for the different stocks studied in different years. There is a tendency for the 1936 data (medium circles) to fall below the 1935 ones (small circles), but the degree of overlap is more striking. There is also a tendency for the snails of any one batch to become lighter and less active as the months go by, but that is unimportant from our present standpoint.

(2) If the lines joining the points for any one animal are studied, it will be seen that they mostly follow the general drift of the belt of points; this is particularly clear in the case of fed animals of high weight and activity. But, in many cases, the lines strike across the general drift. The scatter, then, is not simply due to the fact that measurements made on a great number of snails are included in the diagram, but is caused to a considerable extent by the behaviour of the individual animals. Clearly, the relationship between weight and activity is not very close.

(3) On comparing fed with fasting animals (white and black circles), it is at once seen that the latter never reach a high respiratory rate, and that the relationship between weight and activity is less well marked in their case. On withholding food from an active snail, it drops more or less rapidly towards the left-hand bottom corner of the diagram, where it wanders about irregularly (Fig. 3). This is confirmed if the fed and fasting points of Fig. 2 are separately divided into weight groups and the average oxygen consumption is calculated for each weight group; activity increases more rapidly with increasing weight in the fed animals. It would therefore appear that water content cannot be the main factor determining activity; if it were, the activity : weight relationship should be clearer in the fasting snails, where water uptake is the only means of increasing the weight. Evidently, it is food—or, to be quite accurate, the combination of food and drink—which boosts the oxygen consumption of a snail.

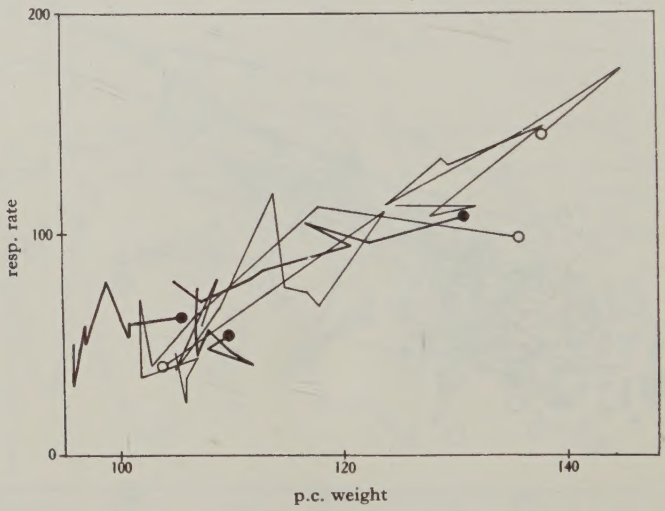

Fig. 3. Day-to-day histories of three fed and three fasting snails. The circle—white for fed and black for fasting animals—indicates the first measurement of each series.

IV. THE SIGNIFICANCE OF AESTIVATION

A typical aestivating snail may be recognized as follows: the animal is wholly withdrawn into its shell, and the opening of the latter is closed by a film of dried mucus, which either runs directly across it or from the rim of the shell to the substratum.

In the course of the present work, and in that of Howes & Wells (1934*a*), animals were often found fully withdrawn, but without any film of dried mucus. Such animals seem to be in the same physiological condition as typical aestivating ones (see Howes & Wells, 1934*a*, Table I), and in the following paragraphs, as in Fig. 1, the two groups are taken together, and described as 'resting'.

Aestivation is generally regarded as a response to unfavourable environmental conditions. Thus Kühn (1914), in contrasting hibernation with aestivation, points out that snails hibernate in autumn even if the conditions are favourable for active life, 'während Beginn und Dauer jeder Trockenstarre ausschliesslich durch äussere Einwirkungen bestimmt werden'. A citation to the same effect from Duval (1930) is given above, on p. 79.

Against this view, Howes & Wells (1934*a*) pointed out that their animals often showed typical aestivation, especially in the troughs of the weight curves; as the weight fluctuations are not due to environmental variables, aestivation evidently occurs, sometimes at least, as a consequence of internal processes.

This conclusion is confirmed by the experiments illustrated in Fig. 1; the snails aestivated from time to time, although the environment was constant. Howes & Wells (1934*a*) kept *H. pomatia* for several weeks in atmospheres so saturated with water vapour that moisture continually condensed on the walls of the containers, and a curious unpublished point from the records of this experiment may here be mentioned: the snails were often found completely withdrawn and, on one or two occasions, they even threw a sheet of mucus (which, of course, could not dry) across the mouth of the shell. The resting state occurred less frequently in moist than in ordinary room air; nevertheless, the fact that it occurred at all in the humidors shows that 'Trockenstarre' is an inappropriate name for the condition.

To bring out more clearly the relation between aestivation and the weight cycles, Fig. 4 was plotted from some of the data used in Fig. 2. The fasting animals were omitted, for the intention is to study the incidence of aestivation under comparatively favourable conditions. A small number of measurements on fed animals, in which the state of the snail was not noted, had also to be left out. The remaining points fall into four groups; it may be recalled that a respiration measurement ends nearly three hours after the snail was taken from the vivarium.

Group 1. In thirty-two cases (black circles), the snail was resting when taken from the vivarium and remained so during the whole respiration experiment. These were animals of low weight and low oxygen consumption; with one exception, all the points lie below the oblique line on the diagram.

Group 2. In 149 cases (white circles), the snail was active (as defined on p. 81) when taken, and remained so during the respiration experiment. All of the resulting points, with only six exceptions, lie above the oblique line.

Group 3. In thirty-seven cases (bisected circles, white over black), the snail was resting when taken but emerged and became active during the respiration experiment. These animals were on the whole of low weight—with few exceptions, the points lie to the left of the vertical line, drawn at 130% of the hibernating weight—but their oxygen consumptions, as one might expect, show a scatter comparable with that of the snails which were active throughout.

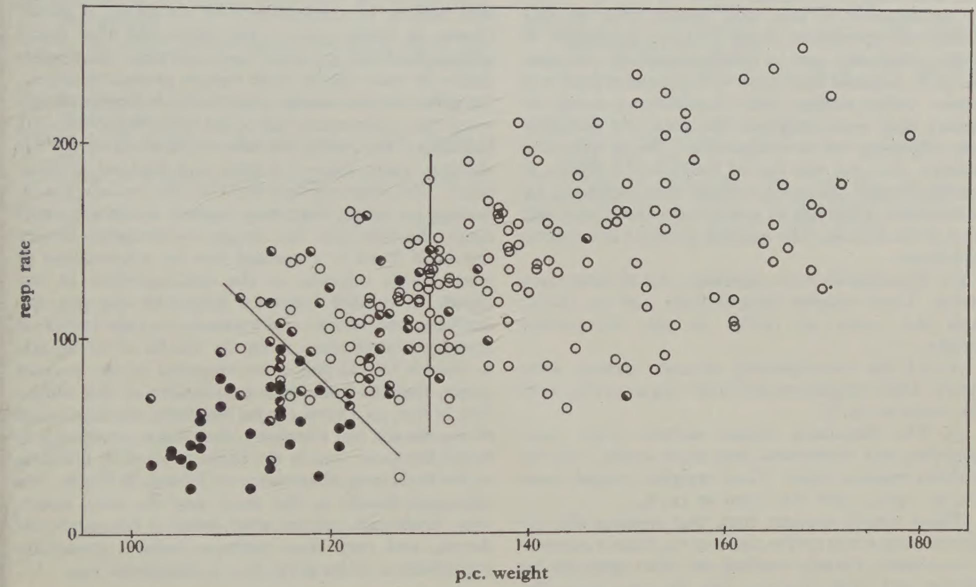

Fig. 4. The distribution of aestivation in fed snails. White circles are 'active' and black, 'resting' snails. Bisected circles are snails which changed their condition during the respiration experiment.

Group 4. In six cases (bisected circles, black over white), the snails were active when taken but withdrew during the respiration experiment. These points are all to the left of the 130% weight line.

The results show very clearly that the tendency to aestivate becomes more and more marked as the bottom left-hand corner of the diagram is approached. Above a weight of 130%, aestivation seldom occurs; below about 115%, it is the rule rather than the exception. The general impression gained by the inspection of a series of records such as Fig. 1 is therefore confirmed.

That aestivation depends, to a large extent at least, on internal conditions is indicated from another angle by the following considerations. According to Duval (1930), the blood of an aestivating *H. pomatia* is much more concentrated than that of an actively creeping one; according to Arvanitaki & Cardot (1932), dilution of the blood in *H. pisana* accompanies the digestive process, which lasts for a couple of days; it therefore appears, if the results on the two species may be put together, that digestion and aestivation are incompatible.

Confirmation of this view comes from an unpublished experiment, made in 1933 by Howes & Wells, which will now be briefly described. A number of *H. pomatia* were kept with abundant food and water. After noting their condition (resting or active), they were weighed, dissected and classified into 'digesting' or 'non-digesting'. In the digesting animals, the crop was full of food; in the others, it contained only the usual reddish fluid, coloured by helicorubin, although in some cases there was still food in the rectum. The animals grouped themselves as follows:

(1) Ten animals were digesting. All of these were active. Their weights ranged from 128 to 170%, with the mean at 148%, of the hibernating weight.

(2) Of the non-digesting animals, sixteen were active. Their weights ranged from 114 to 151%, with the mean at 131%.

(3) The remaining fifteen animals were non-digesting and withdrawn into their shells, with or without mucous films. Their weights ranged from 104 to 146%, with the mean at 121%.

These results suggest, first, that feeding and digestion only occur on the peaks of the weight curve—a conclusion already reached on other grounds by Howes and Wells (1934*a*, Fig. 6)—and secondly, that an animal which has recently fed is unable to aestivate, at least until the digestive processes have gone so far that the crop is cleared of food.

V. CONCLUSIONS

From the available data, laced with a certain amount of speculation, we can draw the following picture of a snail's activity in relation to internal and external variables:

(1) If the snail is kept under reasonably favourable conditions, with plentiful supplies of food and water, it undergoes somewhat irregular weight cycles of considerable amplitude (Fig. 1, lower half). On the weight peaks, it feeds and digests, its blood is consequently dilute, its behaviour is active and its oxygen consumption is high. In the troughs, on the other hand, its digestive organs are resting, its blood is more concentrated, its behaviour is sluggish and its oxygen consumption is low; at these times, it tends to withdraw into its shell and aestivate. The fluctuations are due in the main to its internal processes.

(2) If no food is available, but other conditions are as above, the snail still shows cycles of weight and activity (Fig. 1, upper half), but the weight changes are comparatively small. In the active phases, failing to find food, its metabolic rate never rises to great heights, and we may infer from the results of Arvanitaki & Cardot (1932) that its blood is never very dilute. It spends more of its time in the resting condition than does a fed snail.

(3) If no water is available—e.g. in time of drought—the snail fails to rise out of the first weight trough into which, in consequence of its cycle, it sinks. Howes & Wells (1934*a*, Fig. 6) found that snails offered food but no water spent all their time withdrawn in their shells, their weight gradually falling, for three weeks—except only for an exceptional day when the experiment had to be moved into another building; 'the snails, stimulated presumably by the shaking, crept around a little and nibbled at their food'. But they ate very little on this occasion, and, finding no water, they soon retired into their shells again. In this state, the oxygen consumption is very low, and there is a gradual loss by evaporation of water, with increase in the concentration of the blood. If rainfall follows a period of drought, the snail becomes active and embarks on new cycles; it seems perfectly clear, from the results of Arvanitaki & Cardot (1932) and those reported in the present paper, that the immediate mechanism of this activation is not, as Duval (1930) suggests, the hydration of the tissues, but a sensory stimulation, operating in much the same way as the move to another building in the incidental observation of Howes & Wells. The raindrops knock on the door, and the snail comes out. Hydration follows after, when it has eaten and drunk, and may then perhaps make a secondary contribution to its great rise in metabolic rate.

SUMMARY

1. If *H. pomatia* are kept under conditions of constant temperature, humidity, illumination, food supply and water supply, their daily weight curves show great spontaneous fluctuations of an irregularly cyclical character, with a period of several days. Snails kept without food, but under otherwise identical conditions, show similar fluctuations of smaller amplitude (Fig. 1).

2. A number of measurements of the oxygen consumption of *H. pomatia* were made at different points on the weight cycle. In fed animals, oxygen consumption tends to rise with weight. In fasting animals, the relationship between the two variables is less marked, and the oxygen consumption is always low (Figs. 2, 3).

3. Aestivation tends to occur, even if environmental conditions are favourable, whenever the weight is low (Fig. 4). Feeding and digestion are

apparently confined to the weight peaks, and incompatible with aestivation.

4. The interrelations of internal and external factors in determining the activity level of *H. pomatia* are discussed. In drought, the activity outbursts are inhibited and the snail remains withdrawn, with low weight and oxygen consumption. Rain, after drought, releases the cycles again; the immediate mechanism of activation is not hydration of the tissues, but a stimulation effect.

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STUDIES IN THE MECHANICS OF THE TETRAPOD SKELETON

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(With Fifty-two Text-figures)

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I. GENERAL INTRODUCTION

Although the individual form of the muscles and bones of a tetrapod body is often known with great accuracy, few, if any, comprehensive attempts have been made to regard the whole system as a complete functional unit. The present studies constitute an attempt to consider the whole vertebrate body as a single mechanical system in which the function of the individual muscles and bones is co-ordinated with those of all, or many of, the others.

From a mechanical point of view, the statics of a tetrapod standing at rest present a complicated three-dimensional system, whilst the animal in motion presents even more difficult dynamical problems. Nevertheless, the animal must conform to mechanical principles common to all animate or inanimate systems. For example, although a horse at rest can, by muscular effort, control the degree of support given by an individual leg towards the support of the body, yet the resultant of the thrusts of all four legs must always represent a force equal in magnitude but opposite in direction to the weight of the animal. In the following pages an attempt is made to reduce the living system to its simplest terms and to consider how far the co-ordination of its various parts can be deduced by purely mechanical arguments. As the general type of analysis is probably unfamiliar to some biologists, it may be convenient, at the outset, to make a comparison between the highly complicated living system with a relatively simple inanimate analogue, and to emphasize the main points in which they resemble or differ from each other. For this purpose, a four-legged table with overhung ends forms a convenient starting-point.

Fig. 1 represents a table resting on four legs, each of which is attached to the top (*ABCD*) by a universal joint (*E*, *F*, *G*, *H*). Within each leg is an elastic spring (*L*, *M*, *N*, *O*), and the centre of gravity of the table is assumed to be equally distant from the centre of each leg. So long as the springs within all the legs are identical in respect of length and elastic properties, each leg will carry one-quarter of the weight of the table and there will be no tendency for the table to rotate about either of the diagonals *AC* or *BD*. If, however, the temper of one spring (*L*) be reduced, the latter will shorten and the table will tip towards that leg; in so doing, the spring (*N*) under the diagonally opposite leg will extend and the thrust exerted by it against the table will be reduced. Equilibrium will be re-established when the thrusts of the two springs *L* and *N* are again equal but less than before the change occurred in spring *L*. If, however, the thrusts of *L* and *N* are reduced, those of *M* and *O* must be increased. So long as the legs are vertical and equally distant from the centre of gravity of the table, the general conditions of equilibrium are that the thrust of any one leg must be equal to that of the diagonal leg, and the sum of the thrusts of two ipsilateral legs must be equal to half the weight of the table. Precisely the same arguments apply to the legs of a horse when they are acting as vertical struts (see p. 91), the extensor muscles of the limb being functionally equivalent to the springs within the legs of the table. Further, if in either a horse or table the legs are not vertical and the feet not equally distant from the centre of gravity of the system, it is still possible to define the thrust made by each leg towards the support of the body, by means of equations which are identical in the two cases (§ II).

If the legs of the table are united to the top solely by smooth universal joints the legs can function solely as struts transmitting forces along their mechanical axes only. If all the legs are vertical no horizontal forces are transmitted to the top of the table, but if any leg be inclined to the vertical it will impress on the table-top a horizontal force whose magnitude depends on the amount of weight resting on the leg and on the angle of inclination of the leg's own axis; equilibrium can only exist if this horizontal force is equal but opposite to that impressed on the top by the three remaining legs (see p. 98). If, however, the top of the table is supported by four legs all of which are functioning as vertical or inclined struts, the resultant equilibrium is clearly unstable, since any slight extraneous horizontal force would upset the balance of horizontal forces acting on the top of the table and on each of the legs. Compensation against such forces can be provided by longitudinal (P_1–P_4; R_1–R_4) and transverse (S_1–S_4; T_1–T_4) elastic braces. Any deformation due to an extraneous median longitudinal force will be resisted by the longitudinal braces, and any median transverse force will be resisted by the transverse braces. Similarly, any extraneous horizontal couple acting on the top of the table can be resisted either by an appropriate co-ordination of longitudinal braces, or of transverse braces, or by a combination of both series. In the case of the tetrapod, the braces are provided by the muscles which unite the limbs to the body. All these muscles operate the limb as a lever enabling it to exert, against the body and the ground, forces at right angles to the limb's own mechanical axis (see p. 99).

If the longitudinal braces in Fig. 1 were all equally powerful they would contribute equally towards the resistance of an extraneous median longitudinal force, but if they are not all equally powerful they will, as passive elastic structures, make individual contributions proportional to their power of resisting extension. If, for example, the combined strength of the longitudinal braces on the right side of the table were not equal to that of the left braces, the table would—when an extraneous median longitudinal force is applied to it—be subjected to a couple which must be resisted by the transverse braces. For practical purposes, therefore, the whole of the musculature which acts between the limbs and body of a tetrapod must be regarded as a single functional unit and not as a series of individual muscles acting more or less independently of each other.

In order that the analogy between the legs of a table and of a tetrapod should be more complete, the straight legs of the former may be replaced by angular structures. If two or more loosely articulated segments are to act collectively as an efficient vertical strut, the joints between the individual segments must be prevented from flexing (under the weight of the table top) by suitable braces tending to extend the individual joints. Such braces are roughly equivalent to the muscles operating about the intrinsic limb joints of the vertebrate. The strain placed on these intrinsic braces or muscles depends partly on the postural angles of the limb bones, partly on the weight carried by the limb, and partly on the forces exerted by the extrinsic longitudinal and transverse braces (see § IV). In other words, there must be, in the case of a vertebrate, a very widespread pattern of co-ordination between the whole of the limb musculature, both extrinsic and intrinsic.

So far it has been assumed that the top of the table (head, neck, trunk and tail of the tetrapod) is rigid and therefore able to resist deformation either by its own weight, the thrust of the limbs, or the pull of the longitudinal and transverse braces. In the tetrapod the body is composed of segments and consequently it is necessary to consider the effect of dividing the top of the table into a series of hinged units. If a hinge is present at *ab*, the overhung section *AaBb* will collapse downwards either under its own weight or under the tension exerted by the braces P_1 and P_2. In order to prevent this, the hinge must be restrained from moving by some form of longitudinal brace X_1 acting dorsally across the hinge. Similarly, if there is a hinge at *cd*, the portion (*cABd*) of the table top lying anteriorly to the hinge will tend to move about the hinge under the resultant of all the vertical forces (weight, thrust of the legs and tension of braces) acting on either side of the hinge. This movement must also be restrained by axial braces acting either above (X_2) or below (W) the hinge according to whether the resultant bending forces operate above or below the hinge. Similarly, if the segments of the table top are united together by universal joints, additional braces will be necessary in order to restrain the top from lateral bending and from torsional displacement (see § V).

Sufficient has now been said to illustrate the fact that when a tetrapod is standing at rest with its weight entirely carried by its four feet, the whole of the intrinsic and extrinsic limb muscles, together with those controlling movements of the vertebral column, must be regarded as one functional unit in which the effort of each constituent part is very closely co-ordinated with all the others.

It is now necessary to consider certain features in which the simple mechanical model of the table with its elastic braces differs fundamentally from a living animal. In the case of the table, stable equilibrium is maintained by the elastic braces whose tension depends entirely on their own inherent elastic properties and on the magnitude, direction and point of application of the extraneous forces acting on the table. In the case of an animal, passive braces (ligaments) of this type play a relatively subsidiary role in maintaining stability of the standing posture; effective stability is maintained by muscular tension which is under the control of the animal. If, in the case of the table, one group of braces were to develop spontaneous tension, movements would occur in the system unless either (i) another group of braces developed tension capable of producing effects equal and opposite to those of the first, or (ii) suitable external forces were applied to the whole table. Thus if the retractor group (R_1–R_4) of braces became active the legs would pivot about the feet unless either (i) the protractor (P_1–P_4) braces became active, or

(ii) an extraneous posteriorly acting horizontal force is applied to the top of the table. Clearly, if equilibrium is to be maintained by the effort of internal braces, there must be a definite quantitative relationship between the effort of all the braces concerned. In the case of an inanimate brace or ligament any compensating effort against an external force or against the action of another brace depends on the degree to which it is stretched, but in the case of a muscle the degree of activity is controlled by the more elaborate mechanism provided by its stretch receptors and other reflex mechanisms; as the external strain is increased or decreased so the number of active fibres increases or decreases, thereby enabling the muscle to adjust its effort to the external load without appreciable change of length.

Before proceeding to a more precise consideration of the tetrapod system it is necessary to remember that the muscles of the body and limbs represent mechanical braces of relatively complex morphology. They are not anatomically divisible into separate intrinsic and extrinsic units, since a muscle which extends a knee joint may, at the same time, tend to rotate the limb about the hip. Similarly, a muscle capable of functioning as one of the protractor braces in Fig. 1 may, simultaneously, act also as a transverse brace. The whole of the present studies is, however, based on the fact that so long as the animal is at rest the whole of the musculature can be regarded as a single functional unit whose total action on the limbs or body is such as is expressible in terms of those exerted by the intrinsic springs and extrinsic braces shown in Fig. 1; only in the case of lower vertebrates is it usually necessary to consider rotation of individual limb segments about their own longitudinal axes. Unfortunately there is no generally accepted convention whereby the movements of the vertebrate limb, as a whole, can be defined, although these are somewhat loosely alluded to as extension, flexion, abduction or adduction. In order to avoid confusion a terminology has been adopted for the present work which, although not entirely satisfactory in all cases, enables the movements of a typical tetrapod mammalian limb to be visualized from Fig. 1 without undue risk of ambiguity. If horizontal axes x, x_1 and y, y_1 are drawn parallel and normal to the median longitudinal axis of the body, any muscular effort tending to rotate the whole limb forwards in a vertical plane through kx will be referred to as *protraction*; rotation backwards through the same place (kx_1) being *retraction*. Similarly, any effort tending to rotate the whole limb outwards or inwards in a vertical transverse plane through y, y_1 will be referred to as *abduction* and *adduction* respectively. So far as the whole limb is concerned forces of protraction, retraction, abduction and adduction can only be exercised by muscles whose site of origin lie on the body and therefore lie *extrinsically* to the limb. On the other hand, all muscular effort yielding forces which act solely along the mechanical axis of the limb itself (i.e. along the axis kz in Fig. 1) is due to elements of the muscular system whose sites of origin lie within the limb itself, and which are therefore *intrinsic* in the same sense as are the springs in Fig. 1 (see, however, p. 103). The terms extension and flexion will be restricted to movements occurring at individual limb joints, other than the hips and shoulders, where risk of ambiguity is absent.

It is necessary to remember that the surfaces of attachment of muscles to bones cannot be represented accurately by points on a diagram and that the tensions developed by the muscles are often very large when compared with the weight of the body; consequently it is usually impossible to draw force diagrams which at all faithfully depict the geometrical relationships of an actual limb. From a theoretical point of view these difficulties are not fundamental, but an analysis which takes them fully into account yields a picture which, on account of its geometrical proportions, tends to mask its own more essential features. At the same time, it is essential to remember the great complexity of the living system and to regard the present studies as an indication of a method available for future experimental attack and not as a complete analysis of an extremely intricate study of three-dimensional mechanics.

Until it is possible to submit them to experimental attack many of the more purely biological inferences to be drawn from a mechanical study of the vertebrate body must be regarded as tentative. Although responsibility for any inaccuracies in these and other conclusions rests solely with the author, any value they possess is due in no small measure to suggestions and help from outside sources. In particular, acknowledgements are due to Mr R. A. Hayes, Dr R. H. Brown and Dr H. W. Lissmann, without whose assistance and constructive criticism the work could not have been pursued.

In order to indicate in general terms the type of biological system to which each mechanical diagram is related, I have at times drawn freely from E. E. S. Thompson's *Studies in the Art Anatomy of Animals* (London, 1896). All outline figures must, however, be regarded as highly diagrammatic and not in any way as representations of particular genera or types.

II. THE EQUILIBRIUM OF THE WHOLE ANIMAL IN RESPECT OF EXTERNAL FORCES

From a mechanical point of view the whole animal can be regarded as a series of bony segments (head, vertebrae, limb bones and girdles) freely hinged to each other and each subjected to the downward pull of its own weight and to the forces exerted on it by its neighbours or by the ground. So long as the animal is standing at rest on its four feet, the general condition of equilibrium is that the resultant of all the other external forces acting on the body as a whole or on each of its constituent segments should be equal and opposite to the weight of the whole body or of the particular segment as the case may be.

Starting with a tetrapod (Fig. 3) standing on a horizontal surface, the animal as a unit is exposed to five external forces: (i) the weight of the whole system acting vertically downwards through the centre of gravity of the system; (ii) a reaction from the ground exerted at the centre of pressure of each foot; these reactions all have vertical components and may, or may not, have horizontal components due to transverse or longitudinal thrusts exerted by the limbs against the ground. In addition, there may be, in some instances, horizontal couples tending to twist the foot on the surface of the ground. In order that the body should be in equilibrium there must be no resultant force or couple acting about any one of the three main axes of the system. If such axes are taken through a point on the ground (*G*) vertically beneath the centre of gravity (*C*) of the body (Fig. 3), movements of the animal along the horizontal axes (*XX* and *YY*) and rotation about the vertical (*ZZ*) axis are solely determined by the horizontal forces or couples acting at the feet; rotational stability about the two horizontal axes (*XX* and *YY*) and movements along the vertical axis, on the other hand, are solely determined by vertical forces. If the surface on which the animal is standing is not horizontal the axes of reference can be taken as normal and tangential to the surface of the ground, and the forces acting on the body resolved into components along these axes. So far as the stability of the whole system is concerned, the forces acting normally to the ground can be regarded as a system independent of the forces acting tangentially to the ground.

Distribution of the body weight between the four feet

The ability to stand, on a horizontal surface, with the whole weight of the body carried by the four limbs is characteristic, among living tetrapods, of the Mammalia and of such forms as the chameleons, in which the longitudinal axes of limbs are largely restricted to vertical longitudinal planes passing near or through the shoulders and hips. In most amphibians and lizards part of the weight of the resting animal is carried by the ventral surface of the body: only when the animal is in motion is the whole weight carried by the limbs. In some of the latter types, however, the resting animal can support itself for brief periods with the abdomen out of contact with the ground, and with the four plantigrade feet displaced laterally to unequal extents; such a posture probably represents a more primitive condition than that of a digitigrade mammal and therefore forms a natural starting-point for analytical study. In order that any tetrapod should be in a state of static equilibrium the resultant of the four vertical reactions from the feet must be equal but opposite to the weight of the animal.

This general condition of equilibrium clearly implies that the centre of gravity of the body should lie vertically above a point within a triangle defined by the centres of pressure of three of the feet. For any tetrapod whose four feet are in contact with the ground there are always two such triangles either of which can, from a mechanical point of view, provide adequate support; with the feet in any given positions relative to the centre of gravity of the body the animal can lift either of two particular feet but neither of the other two. The ability or non-ability to lift a particular foot is illustrated in Fig. 4. If the centre of gravity (*G*) lies within the triangle *ABE* the animal can lift either of its hind feet, if within *BEC* it can lift either of the two right feet, if within *CED* it can lift either front foot, and if within *DEA* it can lift either of the left feet; only under highly artificial conditions can an animal lift either of the two limbs lying on the same diagonal. An example of these principles is provided by such an animal as a horse whose centre of gravity lies nearer to the shoulders than the hips; if the axes of all the limbs are vertical, the animal can readily stand with either hind limb flexed and therefore carrying no weight; only after initial protraction of all the limbs can the animal lift either front leg. In a rabbit the conditions are reversed and the animal can in its normal standing posture lift either front limb; it cannot lift either of the hind feet. Clearly, the use of the front limbs for functions other than standing or locomotion depends on the position of the centre of gravity relative to the four feet.

So long as the animal is standing on three feet, the contribution of each towards the support of the body weight is definitely fixed in terms of the weight of the body and position of the feet relative to the centre of gravity. If the generalized type shown in Fig. 5 is standing with its right hind-limb off the ground, the contribution (V_1, V_2 or V_3) made by each of the effective feet can be determined by equations (i)–(iii):

$$V_1+V_2+V_3=W, \qquad \text{(i)}$$

$$V_1x_1+V_2x_2=V_3x_3, \qquad \text{(ii)}$$

$$V_1y_1=V_2y_2+V_3y_3. \qquad \text{(iii)}$$

In these equations the weight of the animal is W; x_1, x_2 and x_3 are the displacements of the feet along the *XX* axis; y_1, y_2 and y_3 are the corresponding displacements along the *YY* axis. Equation (ii) must be satisfied if the animal is not to pitch backwards or forwards about a transverse horizontal axis; equation (iii) must be satisfied if the animal is not to roll about a longitudinal horizontal axis. The values of V_1, V_2 and V_3 obtained from equations (i)–(iii) are, however, too unwieldy to be of much practical interest and, in practice, an animal is normally supported by four legs and not by three, and therefore it always has two possible triangles of support; by modifying the thrust of its limb muscles the animal can distribute the total load between the two triangles in any proportion. The general conditions of equilibrium under such circumstances can be visualized by assuming that the animal in Fig. 4*a*, when supported by the triangle (*ABC*) provided by its right fore, left fore and left hind limbs, applies a downward thrust (V_4) against the ground with its right hind foot. If the animal is not to pitch forward about the diagonal *AC*, the moment of V_4 about *AC* must be compensated by an increased thrust (V_2) from limb *B*, and since the total thrust of all four limbs must remain equal to the weight of the body, the thrusts from the right fore and left hind limbs must both be decreased. For an animal standing on four limbs there must therefore be a system of diagonal co-ordination whereby any change in the activity of one limb is accompanied by a similar change in that of its diagonal fellow, together with a change in the opposite direction in the activity of both members of the other diagonal. This important principle can be expressed quantitatively by considering the equilibrium of the system in respect of vertical and horizontal axes drawn at a point on the ground vertically beneath the centre of gravity of the body (Fig. 5). If the animal is not to pitch backwards or forwards about the transverse horizontal axis or roll about the longitudinal horizontal axis the moments of the foot reactions about these axes must be zero. Equilibrium will exist when

$$V_1x_1+V_2x_2=V_3x_3+V_4x_4, \tag{iv}$$

$$V_1y_1+V_4y_4=V_2y_2+V_3y_3, \tag{v}$$

$$V_1+V_2+V_3+V_4=W. \tag{vi}$$

From these equations the vertical force acting at each of any three feet can be expressed in terms of the force acting at the fourth foot and of the weight of the whole animal. The values so obtained are relatively complex and their real meaning becomes clearer by transferring attention from the generalized tetrapod illustrated in Figs. 4 and 5, to a typical cursorial mammal (e.g. Fig. 6 seq.). In this case $y_1=y_2=y_3=y_4$, and the values for the forces exerted by the right fore (V_1), left fore (V_2) and left hind (V_3) feet become

$$V_1=\frac{W}{2}-V_4, \tag{vii}$$

$$V_2=\frac{W}{2}\left(\frac{x_3-x_1}{x_2+x_3}\right)+V_4\left(\frac{x_1+x_4}{x_2+x_3}\right), \tag{viii}$$

$$V_3=\frac{W}{2}\left(\frac{x_1+x_2}{x_2+x_3}\right)-V_4\left(\frac{x_1+x_4}{x_2+x_3}\right). \tag{ix}$$

will be noted that when a mammal is standing on three legs equally displaced laterally from the median longitudinal axis of the body, one of the limbs supports half of the weight of the body. Further, so long as $x_3>x_1$ there are clearly two limiting and positive values for V_4 which permit of equilibrium, viz. 0 and $\frac{W}{2}\left(\frac{x_1+x_2}{x_1+x_4}\right)$. In the first instance the body is entirely supported by the right fore, left fore and left hind limbs, and in the second instance by the right fore, left fore and right hind limbs. In the latter case, however, the pressure (V_1) on the right fore limb is $\frac{W}{2}\left(\frac{x_4-x_2}{x_1+x_4}\right)$, and stability is only possible when $x_4>x_2$. The degree of control which the animal has over its limbs therefore depends on the positions of the feet relative to the centre of gravity of the body. Equations (vii)–(ix) show that if V_4 be reduced, the thrust (V_2) of the left fore limb must be reduced by an amount which depends on the relative position of all the four feet, whilst that of the left hind foot must be increased by a similar amount. Simultaneously, the thrust of the right fore foot (V_1) must be increased by an amount equal to the decrease in V_4. This general picture is clearly in harmony with that which results from a flexor stimulus applied to one hind limb in either mammals or Amphibia (see p. 93).

An alternative, but very convenient, picture of the co-ordination between the vertical thrust of each of the four limbs of a mammal is provided by a consideration of the right and left limbs acting as pairs (Fig. 6). The resultant of the thrusts of the left fore and left hind feet represents a force equal to half the weight of the animal and acting at a point (*H*, Fig. 6*b*) on the line (*CB*) joining these two feet, the point of action depending on the relative load on the two feet; any increase in the thrust of the left fore foot moves the common centre of pressure of the two feet anteriorly, and any decrease in the thrust of the left fore foot moves the common centre of pressure posteriorly. In order that the whole body should be in equilibrium the common centre of pressure of the two right feet must lie on the line *AD* at a point *I* (Fig. 6*c*) such that the line joining the two centres of pressure (*H* and *I*) passes through *G*, the point at which a vertical line through the centre of gravity of the body meets the ground. Any change which tends to move the common centre of the right limbs anteriorly must be co-ordinated with a change tending to move that of the left limbs posteriorly and vice versa: in other words, any increase in the thrust of one limb involves an increase in that of the diagonal limb and a decrease in both of those on the other diagonal. Alternatively, the state of equilibrium may be defined as that in which any pitching or rolling couple exerted on the animal by the two right limbs is equal but opposite to that exerted by the two left limbs.

On the general principle that an animal is unlikely to stand in such a way as to place unnecessary strain on any individual muscles (see pp. 101 seq.), the extreme limits of variation allowable by equations (vii)–(ix) are unlikely to be observed. If, for any reason, a heavy animal wishes to lift one of its right limbs it may be expected to roll its centre of gravity to the left, thus reducing the thrust required from the remaining right limb. Similarly, any difference between the vertical thrust of the two hind or two fore limbs exposes the vertebral column to longitudinal torsion which must be resisted by the muscles and ligaments of the back (see § V, p. 108); the strain on the latter elements is entirely obviated if the vertical thrust of one hind limb is equal to that of the other whatever be the posture of the feet. This condition is reached in equations (vii)–(ix) when

$$V_4 = V_3 = \frac{W}{2}\left(\frac{x_1+x_2}{x_1+x_2+x_3+x_4}\right)$$

and

$$V_1 = V_2 = \frac{W}{2}\left(\frac{x_3+x_4}{x_1+x_2+x_3+x_4}\right)$$

in other words, when the load is distributed between the front and hind feet inversely in proportion to the average distance of the fore and hind feet from the centre of gravity of the body.

It may be noted that in so far as torsional strain is compensated by vertebral muscles and not by ligaments, an animal can, by means of these muscles, control the vertical thrusts of its limbs against the ground. Alternatively, if the torsional forces exerted by the vertebral column are known, an additional equation of equilibrium is available, and therefore the thrust of each of the four legs can be completely expressed in terms of (i) the animal's weight, (ii) the position of the centre of gravity, (iii) the positions of the centres of pressure of the feet, and (iv) the moment of the tension of the vertebral muscles about the centre of rotation of a vertebral joint.

Bipedalism

Fundamentally similar conditions for static stability apply to bipedal forms. In all such cases there are two effective areas of support (heel and toes) provided by each foot, and these four areas can be treated in precisely the same manner as the four individual feet of a tetrapod. If, by voluntary effort of the extensor muscles of the human ankle (see § IV, p. 102) the centre of pressure of one foot is moved forward towards the toes, appropriate action must be taken by the other limb to move its centre of pressure towards the heel. So long as the centre of gravity falls within the rectangle joining the heels and toes the body can be in equilibrium. An example of diagonal co-ordination between the feet of a human subject is illustrated in Fig. 7.

In the case of a bird standing on one foot, two or more triangles of support are provided by the single foot, and for any given effort on the part of the extensor tendon of one digit the effort required from the remaining digits is determinable.

Comparison of mechanical and physiological data

Regarding an animal from a purely mechanical standpoint it is clear that when a cursorial tetrapod is supported by its four limbs the thrust which any limb exerts normally to the surface of the ground can be varied, between strictly defined limits, without altering either the centre of gravity of the body or centres of pressure of the feet provided that a definite pattern of readjustment is effected in the thrusts exerted by each of the three remaining limbs. Diagonally situated limbs act in unison in the sense that any change in the thrust of one member must be accompanied by a simultaneous change in the same direction of the thrust of the other member; contralateral limbs at the same level of the body act, however, in opposite directions, in the sense that an increase in the thrust of one member must be accompanied by a decrease in the thrust of the other.

The general physiological picture of limb reflexes provides a parallel to that derived from mechanical considerations. Diagonal flexor response to flexor stimulation (which clearly tends to reduce the effective thrust of the limb to which it is applied) was observed in mammals by Sherrington (1906), who also observed an extensor response in both limbs of the other diagonal; comparable phenomena have been recorded for Amphibia by Gray & Lissmann (1940). Similarly, the well-known crossed extensor reflexes between pairs of limbs at the same level of the body conforms to the general mechanical picture. The level of stimulation employed for such observations is usually somewhat drastic, and the results are possibly only comparable to an unstable mechanical situation in which the whole of the weight of the body would be carried on one diagonal pair of limbs. For the maintenance of normal standing postures the necessary co-ordination between the thrusts of the limbs is almost certainly effected by a myotactic mechanism. Liddell & Sherrington (1924) have shown that the tension developed by the extensor muscles of a limb depends reflexly on the external load to which they are subjected. When a right hind limb exerts an increased pressure against the ground, failure on the part of the left fore limb to effect a simultaneous increase would cause the extensor muscles of the latter limb to stretch owing to the downward movement of the left shoulder; this stretch is opposed by the reflex action of the muscles. Similarly, as the load is decreased on the limbs of the other diagonal so the intrinsic activity of their extensor muscles is reflexly decreased. By virtue of this proprioceptive mechanism, each limb acts as an elastic structure exerting a variable thrust equal and opposite to that to which the body is subjected by its own weight and by the thrust of the other three limbs.

The mechanical analysis as given in this paper assumes that when the thrust of one limb is changed the animal does not displace the centre of gravity of the body. If such a change occurs the mechanical picture alters accordingly. The degree of movement of the centre of gravity can only be determined experimentally; actual tests of the behaviour of tetrapods in this respect being impracticable under existing world conditions, observations derived from human subjects (when supporting the body on hands and knees, the elbows being partially flexed) are of some interest. In order to prevent changes in the position of the centres of pressure of the limb, the weight was carried on the surfaces of the knees and on digits fully flexed. Each limb rested on a spring balance.

(i) For subjects standing normally without conscious bilateral asymmetry of effort, the weight of the body was distributed equally between the right and left pairs of limbs to within 5% of the total body weight, although the posture of individual limbs might vary considerably. Each subject tended to be slightly biased to one side in the sense that a slight excess of weight tended to be carried by one particular side.

(ii) The predictable redistribution of weight between the three remaining limbs was invariably observed when the effort of the fourth limb was consciously changed (Fig. 8).

(iii) For symmetrically placed pairs of limbs (i.e. $x_1=x_2$, $x_3=x_4$) the distribution of weight between the fore and hind pairs of limbs was unaffected, although the range of effort of any individual limb might vary by 25% of the total body weight.

From these data it may be concluded that a fairly wide range of posture and of individual limb effort can be exhibited by a tetrapod without altering the position of the centre of gravity of its body. It must be remembered, however, that a voluntary movement of the centre of gravity by simultaneous protraction or retraction of all its limbs enables an animal to vary the distribution of the weight of its body between the fore and hind limbs, while abduction or adduction of all the limbs enables it to redistribute the weight between the limbs on the two sides of the body.

Equilibrium of the body in respect of tangential forces acting at the feet

As pointed out on p. 91, the rotational stability of the whole body about an axis normal to the ground and translocatory stability along the two tangential axes are determined by such tangential forces as may be acting at the feet; so long as the whole animal is standing freely at rest on a horizontal surface the resultant of these forces must be zero. As will be shown in § II, the magnitude and direction of the tangential forces acting at any one foot are under the control of the animal, and in practice the animal probably adjusts their values to such levels as will not place an undue strain on any of the muscles of any one of the four limbs. Any change in the magnitude or direction of the tangential force acting on one foot must, however, be compensated by an equal and opposite change in the resultant of the tangential forces acting at the other three feet. As can be seen from Fig. 9, this adjustment can, in a generalized tetrapod, be made in a variety of ways and does not involve any fixed pattern of limb co-ordination comparable to that involved by a change in the vertical thrust. In the case of the Mammalia, however, any substantial change in the longitudinal component acting at one foot normally involves an equal and opposite change operating at the other ipsilateral foot.

If a tetrapod is standing on an inclined surface or is, when on a horizontal surface, subjected to an external horizontal force, the resultant of the tangential forces acting between the feet and the ground must be equal and opposite to the tangential component of the weight of the body or to the applied external force as the case may be. In the case of a mammal (Fig. 10) standing freely on an inclined (α) surface, the tangential component of the weight is $W \sin \alpha$ and the component normal to the surface is $W \cos \alpha$, and these forces can be assumed to act at a point (G) on the ground vertically beneath the centre of gravity (C). In order that equilibrium should exist, the combined centres of normal pressure of all four feet must act upwards at G, and exert a total force normal to the ground of $W \cos \alpha$, whilst the resultant of the tangential forces acting at the feet must be equal and opposite to $W \sin \alpha$ also acting through G. If the posture and loading of the feet on the two sides of the body is symmetrical, the normal component ($W \cos \alpha$) of the weight of the body will be distributed between the front and hind feet in a proportion inversely proportional to the distances of the centres of pressure of the feet from the point G (Fig. 10). On the other hand, the distribution of the tangential force (required to neutralize the tangential component of the weight) between the front and hind limbs is, from a mechanical point of view, immaterial; equilibrium can exist so long as half of the total force required is provided by each ipsilateral pair of limbs; if, however, the limbs are all acting as struts (see p. 98), as in Fig. 10, the total tangential force will be divided between the feet in the same proportions as the forces acting normally to the ground. In the absence of claws or other prehensile devices the tangential force exercised by each limb against the ground is opposed by friction and must not exceed a critical value relative to the force which the limb exerts normal to the ground.

In the case of an animal (Fig. 11) standing on a horizontal surface and subjected to an external horizontal force in addition to the weight of the body, the conditions are essentially similar to those of an animal standing on a slope. If the external force tends to pull the body forwards, the effective centres of pressure of the two right and two left feet both move forward, thereby increasing the strain on the front feet and decreasing that on the hind feet. To counteract this effect, the animal frequently protracts its limbs (Fig. 11*b*), thereby moving the centre of gravity of the body posteriorly relative to the feet.

for Italy and Austria together. Palffy, all smiles, was applauded at the Fenice theatre that evening, when this short-lived honeymoon reached its culmination, with *vivas* for Manin, Pio Nono and the Emperor Ferdinand. Only the Croat soldiers failed to catch the enthusiasm of the hour.[1]

At Milan there was no such momentary reconciliation between Governors and people. And even in Venice there were many Italians who, though they rejoiced, neither expected nor desired this phase of things to last, and who were resolutely determined not to fall out of the great national movement of Italy for the bribe of Austrian Home Rule. Manin had made up his mind in prison, and he knew no shadow of turning. Besides, there was less than no security for these concessions, even if Venice were base enough to accept them. In two months, he told his friends, the Government would go back on its promises, as it had on those of 1815. For all they knew, said Manin, Milan might already be in arms. It was their duty to support the movement in the rest of Italy and expel the Austrians from Venice.[2]

The next two days (March 20-21) passed uneventfully. The Civic Guard daily increased in numbers and improved in discipline, and were allowed by Palffy to join or replace the white-coats at one strategic point after another, sharing with the regulars the guard on the Piazza and the Riva degli Schiavoni which joined the Piazza to the Arsenal. The wearing of tricolour ribbons became a fashion which the Government could neither authorize nor prevent. Otherwise there was little outward change since the 19th, but the political atmosphere

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[1] *M.C.V.*, *Cavedalis MSS.*, i. pp. 87-101. *Raccolta*, i. pp. 5-7, 12-13. *Rovani*, pp. 32-33. *Cusani*, pp. 19-20. *Gazz. Ven.*, March 19. *Carte Segrete*, iii. p. 229. *Blue Book*, ii. p. 268. *Steinbüchel*, p. 18. *Helfert*, i. p. 339. *E and F.*, pp. 340-341, is wrong only as to the hour of the Trieste steamer's arrival at Venice.

[2] *Radaelli*, *Manin*, p. 55. *Helfert*, i. p. 340.

answered that he could not conceive the fall of the Austrian Government as possible, that the Venetians were unused to self-sacrifice, and that Manin had no right to risk the lives of millions.[1]

But Manin did not wait for Municipal or academic approval before making his plans and preparations for the *coup*. By the night of the 21st he had his men engaged and their parts assigned for the seizure on the morrow of the Arsenal and of the four cannon on the Piazza. The conspirators were his personal friends like Degli Antoni and a few naval officers like Paolucci. The Italian troops composing part of the garrison in the Arsenal had been warned in general terms. Some of the officers of the Civic Guard had offered their services, but others, including the Commandant Mengaldo, regarded the enterprise as 'madness.'[2]

When, too late, Palffy asked for a conference with Manin, he replied:

> 'Tell the Governor he would not yield to my demands when he might have done so. Now I would ask things he could not grant. I have never deceived anyone, and I won't begin now.'[3]

On the evening of the 21st, Pincherle propounded his question to the Municipal Council for the last time.[4] It was impossible any longer to ignore what might happen next day, and his colleagues on the Municipality asked him to go to Manin and sound him as to his plans. He returned with the message that Manin had indeed

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[1] In April Tommaseo wrote to Manin, 'You know that from the 21st of March I have foreseen the difficulties and that I summed them up in these words—We have a people unused to arms and not accustomed to sacrifice.' *M.C.V.*, *Manin MSS.*, 4005. See also *E. and F.*, p. 363. *Tom. e Cap.*, iii. pp. 228-232. *Tommaseo*, iii. p. 321. *La Forge*, i. pp. 257-258, 261.

[2] *Radaelli*, p. 48. *Marchesi*, pp. 110, 114-115. *Federigo*, p. xxii.

[3] *Federigo*, p. xx.

[4] Pincherle was not officially invited to join the Council till March 22 (*Errera*, p. 435), but he and five others had been unofficially asked to strengthen the existing Municipality as far back as the 19th and had done so. Manin had been asked but had declined, in order to keep a free hand. *M.C.V.*, *Manin MSS.*, No. 3811. *La Forge*, i. p. 261.

For such an animal (e.g. Fig. 11*b*) the resultant of the forces acting on the body normally to the ground is not zero but constitutes a couple which is equal and opposite to that due to the extraneous horizontal force and the friction at the feet; in this sense the animal uses the weight of its body to counteract the effect of the extraneous force.

Similarly, the application of a suitable extraneous force to an animal standing on a slope may elicit the posture characteristic of the animal when standing freely on a horizontal surface (Fig. 12). In all cases the natural posture is such that the distribution of weight between the front and hind feet conforms closely to that characteristic of an animal standing freely on a level surface.

Scansorial types

An ungulate animal can only stand on an inclined surface if two conditions are satisfied: (i) adequate friction operates at the feet, (ii) a vertical line through the centre of gravity of the body falls within the quadrilateral of the feet. In practice, the animal usually adopts one of two characteristic postures; (i) the axes of the limbs are retracted while that of the body is parallel to the slope (Fig. 12*c*), (ii) the axis of the body is held horizontal by flexure of either the front or hind limbs according to the direction of the slope (Figs. 10, 12*d*). From a systematic point of view, animals living in an environment free from steep slopes are characterized by long ungulate limbs, whilst those adapted to scansorial, or other conditions involving steep slopes, typically possess elongated bodies, with short limbs usually ending in prehensile digits or claws. All these postural and morphological features follow naturally from a consideration of mechanical arguments. Starting with an animal standing with the axes of all its limbs vertical, with its centre of gravity at C_1 (Fig. 13), the total vertical load on the two front feet must be $W\frac{b}{a+b}$ and that on the two hind feet $W\frac{a}{a+b}$, where a and b are the distances of shoulders and hips from the centre of gravity.* If the surface on which the animal is standing be now inclined downwards at an angle α, the line of action of the weight of the body lies in a vertical transverse plane which cuts the ground at a distance from the front feet (AG_2, Fig. 13*b*) equal to $a-(h-c)\tan\alpha$, where h is the length of the legs and c is the distance of the centre of gravity below the main axis of the body. In order that the common centre of pressure of all the feet should lie vertically below C_2 the distribution of weight between the front and hind feet must be

$$V_1+V_2=W\frac{b+(h-c)\tan\alpha}{a+b}, \qquad \text{(x)}$$

$$V_3+V_4=W\frac{a-(h-c)\tan\alpha}{a+b}. \qquad \text{(xi)}$$

Similarly, if the animal is standing on an ascending slope

$$V_1+V_2=W\frac{b-(h-c)\tan\alpha}{a+b}, \qquad \text{(xii,}$$

$$V_3+V_4=W\frac{a+(h-c)\tan\alpha}{a+b}. \qquad \text{(xiii)}$$

Clearly an ungulate cannot stand (with the axes of its limbs normal to the ground) on a descending slope the tangent of whose angle of inclination is greater than $\frac{a}{h-c}$, or on an ascending slope whose tangent is greater than $\frac{b}{h-c}$.* In order to stand on steep slopes with the axes of the limbs in their normal posture vertical to the axis of the body, it is necessary to have a long body $(a+b)$, short legs (h) and a low centre of gravity (c). The more these conditions are satisfied the less danger is there of the animal tipping backwards or forwards about its front or hind feet; the amount of weight $\frac{(h-c)\tan\alpha}{a+b}$ which the slope automatically transfers from front to hind limbs or vice versa decreases likewise. As shown by Fig. 13*c*, however, this transfer of weight is avoided if the animal adopts a vertical posture of its limbs; under these conditions $AG_3=AG_1$ and consequently the distribution of weight between front and hind limbs is that characteristic of the animal when standing on a horizontal slope with its limbs in the normal vertical position relative to the body (Fig. 13*a*).

On very steep slopes the typical position of rest, particularly of carnivores, is characterized by a horizontal posture of the back (Fig. 10). The mechanical advantage of this position is illustrated by Fig. 13*d*. The animal is assumed to be originally standing on a downward slope (α) and, keeping the axes of the fore limbs normal to that of the body, to have flexed the hind limbs and allowed the body to pivot backwards about the front feet through an angle γ; as before, the centre of gravity of the body is at C_4 which lies a distance c ventrally to the axis of the body and at distances a and b from shoulders and hips respectively. If the length of the front limb

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* In Fig. 13, $a=b$ and the distribution of weight is shown numerically.

* If the axes of the limbs are not vertical equations (xii) and (xiii) become

$$V_1+V_2=W\frac{b+x_4-(h-c)\tan\alpha}{a+b+x_1+x_4}, \qquad (\text{xii}a)$$

$$V_3+V_4=W\frac{a+x_1+(h-c)\tan\alpha}{a+b+x_1+x_4}, \qquad (\text{xiii}a)$$

where x_1+x_4 are the distances by which the fore and hind limbs are displaced outwards from the shoulders and hips respectively. The tangent of the critical angle of the ascending slope is therefore increased to $\frac{b+x_4}{h-c}$; a retraction of the hind limbs in accordance with this principle can be frequently observed in the case of a dog (Fig. Rademaker, Abh. 98).

be h, the distribution of the body weight between the front and hind limbs is

$$V_1+V_2=W\left[1-\frac{(h-c)\sin(\gamma-\alpha)\cos\gamma+a\cos(\gamma-\alpha)\cos\gamma}{(a+b)\cos\alpha}\right], \qquad \text{(xiv)}$$

$$V_3+V_4=W\left[\frac{(h-c)\sin(\gamma-\alpha)\cos\gamma+a\cos(\gamma-\alpha)\cos\gamma}{(a+b)\cos\alpha}\right]. \qquad \text{(xv)}$$

If $\gamma=\alpha$

$$V_1+V_2=W\frac{b}{a+b}, \quad V_3+V_4=W\frac{a}{a+b}.$$

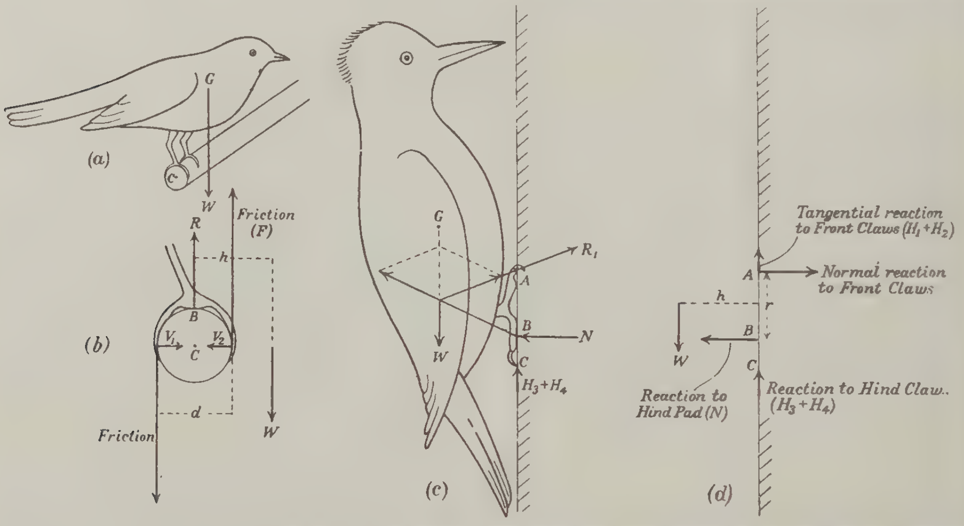
G
(a)
C
W
R
Friction
(F)
h
B
(b)
V_1
V_2
C
d
Friction
W
G
R_1
A
B
N
C
W
H_3+H_4
(c)
Tangential reaction to Front Claws (H_1+H_2)
A
Normal reaction to Front Claws
h
r
B
W
C
Reaction to Hind Pad (N)
Reaction to Hind Claw.. (H_3+H_4)
(d)

Fig. 15. *a*, *b*. Equilibrium of forces acting on a perching bird when the line of action of the body weight does not pass through the centre of the perch. The perch exerts at B an upward reaction (R) equal to the weight of the animal, and the body is subjected to a forward couple Wh; W is the weight of the bird and h is the distance between the lines of action of R and W. By activity of their flexor muscles, the digits exert equal and opposite pressures (V_1, V_2) normal to the surface of the perch: the magnitude of these pressures must be such that they enable the frictional couple (Fd) to be equal and opposite to that due to the weight of the body (d=the diameter of the perch).

c, *d*. Equilibrium of forces acting on a bird attached to a vertical surface of a tree. The couple (Wh) due to the weight is balanced by a pull exerted by the anterior digits and a push exerted by the posterior digits, both acting normally to the surface of the tree. The forces acting tangentially to the tree surface (H_1, H_2, H_3, H_4) are together equal to the weight of the bird. The short legs of climbing birds is an adaptation reducing the strain which falls on the muscles of the leg. The precise distribution of the forces over the surface of the foot is somewhat hypothetical; in life the tail may exert a pressure against the tree. R_1=reaction to front claws; N=normal reaction to posterior digit; H_3 and H_4=tangential reaction to hind claws; W=weight of animal; G=centre of gravity.

In other words, if the animal stands with its back horizontal, the distribution of weight between the front and hind limbs is the same as when the animal is standing on a horizontal surface with the axes of its limbs vertical. It may be noted that if $\gamma=0$, equations (xiv) and (xv) reduce to equations (x) and (xi). As pointed out in § IV, this type of adaptation to an inclined surface is usually accompanied by a transfer of the centre of pressure of the hind feet from the toes to the heel (see Fig. 10) in order to avoid placing an undue strain on the extensor muscles of these joints. The fundamental principle, however, remains unchanged.

If claws or similar prehensile structures are present on the feet the range of posture on an inclined surface is very greatly increased, since the centre of gravity need not fall within the quadrilateral of the feet and there need be no limiting ratio between the tangential and vertical forces acting at the feet. The general conditions of an equilibrium of this type can be illustrated by a lizard at rest on a vertical but rough surface (Fig. 14). In this case the hind limbs press downwards and inwards against the wall whilst the fore limbs pull downwards and outwards, the resultant of the reactions of these two forces being equal and opposite to the weight of the body. When in equilibrium, the animal is subjected to two equal and opposite couples, one due to the weight of the body and the tangential vertical forces acting at the feet and the other due to the forces acting at the feet normally to the wall. For such conditions also a long low body represents an adaptive feature. Similar conditions apply to a bird of the woodpecker type (Fig. 15, *c*, *d*). In the case of a bird standing on a horizontal perch (Fig. 15 *a*, *b*) any vertical couple due to the weight of the body is compensated by a frictional couple exerted by the perch against the digits.

SUMMARY OF §§ I AND II

1. The general conditions under which a tetrapod can use any particular limb for purposes other than the support or propulsion of the body depends on the positions of the four feet relative to the centre of gravity of the body.

2. When a cursorial tetrapod is standing on four legs, the contribution which any one limb makes towards the support of the body can vary within limits which can be defined in terms of (i) the weight of the body, (ii) the position of the centres of pressure of the four feet relative to the centre of gravity of the body. If the thrust of one limb is known that of each of the other three can be calculated.

3. If the vertical thrust of any one limb be increased, there must be a simultaneous increase in the thrust of the diagonally situated limb and a decrease in the thrusts of both feet situated on the other diagonal. The reflex myotactic response of mammalian extensor muscles to mechanical stretch provides an adequate mechanism for ensuring effective co-ordination between the vertical thrusts exerted by each of the four limbs. The physiological response of all the limbs to flexor stimulation of any one of them conforms to the mechanical requirements for stability; it probably represents an extreme instance of readjustment of limb thrusts.

4. In bipedal forms a quadrilateral of support is provided by the heel and toes of the two feet: the conditions of stability are essentially the same as in tetrapods.

5. If a tetrapod stands on an inclined slope (in a posture similar to that normally adopted on a horizontal surface) the extent of the resultant redistribution of weight between front and hind feet is inversely proportional to the relative length of body and limbs. A long low body is a mechanical adaptation to a scansorial habit. The typical postures adopted by tetrapods when standing on steep slopes (viz. either with the axes of the limbs retracted or the back horizontal and one pair of limbs flexed) conform to the principle that the distribution of the total weight of the body between the various limbs should approximate closely to that characteristic of the animal standing in a normal posture on a horizontal surface. The same principle applies to the posture adopted by an animal when exposed to restraint by an extraneous horizontal force.

III. THE FUNCTION OF LIMBS AS STATIC SUPPORTS

From a mechanical point of view a tetrapod can be regarded as an elongated body or beam supported by four legs each of which is attached to the beam by (i) a freely movable joint and (ii) a number of extrinsic muscles. For immediate purposes the vertebral beam will be regarded as a rigid unit and each limb as a telescopic structure capable, by internal muscular effort, of exerting forces along its own mechanical axis, i.e. along the line joining the centre of rotation of the hip or shoulder joint to the centre of pressure of the foot against the ground. Under such conditions each limb can exert on the body (i) a pressure applied by the head of the limb against the centre of rotation of the hip joint or against the serratus muscles, (ii) a tension acting at the site of origin of each of the muscles operating between the limb and the body. Each of these forces can be resolved into three components: (i) vertical, (ii) longitudinal horizontal, and (iii) transverse horizontal. In order that the vertebral beam (body) should remain at rest the following conditions must be satisfied: (i) the sum of all the vertical forces exerted by the limbs must be equal but opposite to the weight of the body, (ii) the sum of the longitudinal horizontal forces must be zero, (iii) the sum of the transverse horizontal forces must be zero, (iv) there must be no residual couple tending to rotate the beam about any of its three major axes. Precisely similar conditions apply to each limb except for the fact that the reaction from the ground represents an additional force acting on the system. The whole system and each of its five component parts clearly represent a relatively complex study in three-dimensional statics. If, however, attention be focused on typical mammalian limbs rather than on the more generalized conditions found in lower vertebrates, the problem can be very greatly simplified without serious limitation in the application of the results.

From a mechanical point of view the typical mammalian limb has three main advantages: (i) the mechanical axes of both ipsilateral limbs lie in approximately the same vertical plane, (ii) the forces tending to displace the limbs or body backwards or forwards are considerably greater than those tending to displace them laterally, and (iii) forces tending to rotate the limb about its longitudinal mechanical axis can be neglected so long as the animal is at rest. Consequently, it is possible to analyse the mammalian system very largely in terms of vertical and horizontal forces all acting in two longitudinal vertical planes. This treatment yields a mechanical picture of the whole system which, although relatively simple, nevertheless leads to the same fundamental conclusions as those derived from the much more complex three-dimensional system presented by the limbs of the lower vertebrates. Further mechanical simplifications can be effected by taking advantage of the fact that the principles whereby a limb is maintained in equilibrium with its own weight are fundamentally the same as those which maintain its equilibrium with the weight of the body (see Appendix I, p. 112); by ignoring the weight of the limb, a much clearer conception of limb function can be obtained and, if a complete picture is required, it is always possible to superimpose that derived from a consideration of the limb's own weight.

So long as the above simplifications are borne in mind each limb of a mammal, standing at rest, can be regarded as a system in equilibrium with three forces: (i) the force exerted by the body against the head of the limb at its effective centre of articulation with the body, (ii) the tensions exerted on the limb by the extrinsic muscles which unite the limb to the body, and (iii) the reaction of the ground against the foot. If the resultant of all the extrinsic muscular tensions is zero, the limb acts as a strut transmitting forces along its own mechanical axis only. If, on the other hand, the resultant tension of the extrinsic limb muscles is not zero, the limb exerts forces at right angles to its own mechanical axis and, to this extent, functions as a lever. During the earlier part of the ensuing discussion the whole musculature of the limb will be regarded as having the functional components, one being solely concerned with the operation of the limb as a strut and the other with its operation as a lever. Subsequently, the action of definitive muscles in each or both of these capacities will be considered.

Function of the limb as a longitudinal strut

In the simplest mechanical case (Fig. 16) all four limbs function as vertical struts in the sense that the centre of pressure of the ground against each foot lies vertically beneath the centre of rotation of the hip or shoulder. As shown in § I, the vertical thrust from any individual limb can, by internal muscular effort, be varied between certain well-defined limits which depend on the relative distances of the front and hind feet from the centre of gravity of the body. In every case, however, the resultant of the external forces acting on each limb and on the body must be zero if the whole system is to remain in equilibrium.

In a great many postures, however, one or more limbs may be inclined to the vertical to such an extent that the vertical line of action of the body weight acting at the hip or shoulder falls outside the surface of the foot (Fig. 17). If such a hind limb is to act as an efficient inclined strut its axial thrust must be such as will produce a vertical upward force at the hip equal to the weight of the body acting at that joint. If this weight be W_4, the axial thrust of the limb must be $W_4 \sec \alpha$, where α is the angle of inclination of the limb's axis. Such an axial thrust will, however, exert at the hip a horizontal force $W \tan \alpha$, and a horizontal force of equal value but of opposite direction will be exerted by the foot against the ground. Such a limb can only be in equilibrium if the body exerts against the head of the limb a compensating horizontal force ($H_4 = W_4 \tan \alpha$ (Fig. 17*a*)) and if, at the same time, the horizontal component of the limb's thrust against the ground is opposed by an equal and opposite tangential resistance (e.g. static friction) from the ground. It may be noted that when a limb is supporting the body in the capacity of an inclined strut, the head of the limb is subjected to two equal and opposite turning forces, one attributable to the weight of the body and the other to a horizontal force exerted on the head of the limb by the body. Similarly, the limb is subjected at its foot to two equal but opposite turning forces, one due to the reaction of the ground to the weight of the body and the other to friction acting between the foot and the ground.

A simple case in which all four limbs function as struts is shown in Fig. 18. Since each limb is assumed to be carrying one-quarter of the total body weight, the right hind and left fore limbs exert anteriorly directed forces against the body each equal to $\frac{1}{4}W \tan \alpha$, where α is the angle of inclination of the limb to the vertical; the horizontal forces exerted by the left hind and right fore limbs are directed posteriorly and also each equal to $\frac{1}{4}W \tan \alpha$. The resultant horizontal thrust of all the limbs against the body is zero and the latter is therefore in equilibrium. So far as any one limb is concerned, the horizontal component of its own axial thrust is equal and opposite to the resultant of all the horizontal forces exerted by the remaining limbs on the body. The right side of the body is under compression whilst the left side is under tension; in other words, the hips are exposed to a longitudinal horizontal couple (H_3, H_4, Fig. 18*d*), whilst an equal but opposite couple (H_1, H_2) acts on the shoulders. As will be shown later (§ V), these couples must be compensated by activity of the vertebral muscles. For the time being, it may be noted (Figs. 18 *b*, *c*) that as in this particular example the load on each foot is equal to that on the contralateral limb (at the same level of the body) the vertebral column is free from torsional stress (see p. 108, § V).

If the two left limbs in Fig. 18 were to adopt the posture already adopted by the two right limbs the system would still be in equilibrium, since the combined anterior thrust imparted to the body by the hind limbs would be equal and opposite to the posterior thrust of the front limbs and vice versa. Neither hips nor shoulders would be exposed to turning couples, nor would the body be exposed to a lateral bending couple: on the other hand, the neutral axis of the vertebral column would be compressed.

It is clear that a limb can, in the capacity of an inclined strut, contribute effectively towards the support of the body provided that (i) the horizontal component of the axial thrust applied to the body by means of the limb's own intrinsic extensor muscles is equal but opposite to the resultant horizontal force exercised on the body by the other three limbs or other external agencies, (ii) friction, equal in value to the horizontal force exerted by the body against the head of the limb, acts at the foot. Any change in the distribution of the weight resting on a limb alters the horizontal thrust which must be forthcoming from the other limbs in order to sustain the limb as a strut.

Limbs as lateral struts

The ability of a limb to act as a lateral strut depends on precisely the same principles as determine its action as a longitudinal strut. An example of transverse struts is shown in Fig. 19, which also indicates one of the equilibrium conditions applicable to the pectoral limbs of many reptiles and to firmisternal Amphibia. It will be noted that the equilibrium of the limbs when acting as inclined lateral struts depends on the ability of the shoulder girdle and body to resist transverse compression. Conversely, in arciferous forms compression of the thorax by laterally displaced limbs can usually only be avoided by using these limbs as levers operated by their pectoral musculature.

In typical cursorial mammals the power of a laterally displaced limb to support a substantial amount of the body weight is often limited, but since some degree of lateral inclination of the limb is possible, transverse forces will operate between the limbs and the body. So far as a limb acts as a strut any transverse horizontal turning couple, due to the weight of the body, must be compensated by (i) a transverse horizontal force, due to the action of the other three limbs, applied by the body to the head of the limb, and (ii) transverse friction acting at the feet. In the case of the body, transverse and longitudinal horizontal couples must be considered together. Any unbalanced horizontal longitudinal couple due to the limbs acting as longitudinal struts will tend to rotate the body about its vertical axis; this can, however, be compensated by a suitable horizontal transverse couple due to the limbs acting as transverse struts. An example is given in Fig. 20.

The operation of the limbs as struts involves no differential activity on the part of the muscles by which the limb is attached to the body. If, however, the whole of this musculature were free from tension the resultant equilibrium of the hip and shoulder joints would be unstable in the sense that it would be upset by very small extraneous forces. During life, the whole of the limb musculature displays a definite degree of tonic contraction, but so long as a limb is acting as a strut the rotary effect of any one group of muscles tending to rotate the limb about the hip or shoulder is opposed by the equal and opposite effect of an antagonistic group; the effect of all the rotary muscles is, under such conditions, restricted to a compression of the head of the limb against the body along the mechanical axis of the limb, the resultant turning effect of all the muscles being zero.

If the body is exposed to an extraneous horizontal force, displacement in respect to the limbs can only occur if one or other group of rotary muscles is stretched. In this case the muscles exert a turning action on the limbs tending to oppose the action of the extraneously applied force; the limbs then act as levers. Similarly, if the horizontal force exerted on the head of a limb by the body is not equal but opposite to the horizontal component of the axial thrust from the limb itself, the difference must be made good by the limb's action as a lever. Difference in longitudinal horizontal forces must be compensated by longitudinal protractor or retractor muscles and differences in transverse horizontal forces by abductor or adductor muscles.

The limb operating as a lever

When a muscle, by which a limb is united to the body, is under tension it tends to rotate the former about its proximal joint and enables the foot to exercise, against the ground, a force at right angles to the limb's own mechanical axis. In such circumstances the limb can be regarded as a lever whose fulcrum is represented by the centre of rotation of its proximal joint.

The mode of action of such a muscle is illustrated in Fig. 21*a*, in which a protractor muscle is developing tension between its origin (O) on the body and its point of attachment (A) on the limb. The tension (T_B) exerted on the body at O can be resolved into components normal (OP) and along (OQ) the line (OH) joining the effective point of origin of the muscle to the centre of rotation of the hip. The force acting along this line can be replaced at H by a force ($HR=OP$) acting at right angles to OH and by a force ($HS=T_B$) acting parallel to the line of action of the muscle. The two forces OP and HR constitute a couple acting on the body and tending to rotate its anterior end downwards, whilst the two forces HS and T_L constitute a couple acting on the limb and tending to rotate the head of the limb backwards and the foot forwards. The moment of the couple acting on the body is equal but opposite to that acting on the limb, whilst both are numerically equal to that of the muscle's tension acting about the joint (see legend to Fig. 21). The application of these equal and opposite couples to the body and limb is the fundamental action of all muscles which act between the body and limbs, for, by adjustment of their tension the limb can be stabilized either against a turning action due to the weight of the animal or of a horizontal force applied to the head of the limb by the body. For a limb subjected at its proximal end simultaneously to a vertical force (V_4) due to the weight of the body and a horizontal force (H_4) due to a thrust exerted by the axis of the body, equilibrium will exist when the sum of the moments of these forces about the foot is equal but opposite to that of one (Tz) applied by the limb's own extrinsic muscles,

$$V_4 l \sin\alpha + H_4 l \cos\alpha = Tz,$$

where V_4 = vertical load on the limb, l = length of the limb and α is its angle of inclination to the vertical, H_4 = horizontal thrust of the body against the hip or shoulder, T = tension of the muscles operating between the body and limb, and z = working distance of muscles from the joint. Clearly, if

$H_4 = -V_4 \tan\alpha$, the limb acts as a strut, but for any other value of H_4 equilibrium is dependent on the limb operating as a lever. Similarly, as long as the vertical load on a limb remains fixed, any alteration in the tension of a protractor or retractor muscle alters the value of the longitudinal friction acting at the foot and the horizontal longitudinal thrust which the head of the limb exerts against the body. Precisely similar considerations apply to limbs acting as transverse levers when operated by adductor or abductor muscles. So far as longitudinal horizontal forces are concerned, control is exercised by the protractor and retractor muscles of the hips and shoulders; protractor muscles exert posterior forces on the body and retractor muscles exert anterior forces. In each case corresponding frictional resistance must act against the foot.

The total vertical force exerted by a limb against the body or ground is the sum of those due to the limb acting as a strut and as a lever; similarly, the total horizontal force exercised against the body and the ground is also the sum of the effects of the limb as a strut and a lever. The vertical leverage of the limb is, however, usually comparatively small so that the main strain falls on the axis of the limb. On the other hand, the muscles which unite the limb to the body may be regarded as the only mechanism whereby the animal can influence the horizontal forces acting on the body without disturbing the distribution of weight between the various feet. From a morphological point of view, the division of the limb musculature into two separate units (one which is solely concerned with the maintenance of forces acting along the mechanical axis of the limb and the other with horizontal forces acting against the body and against the ground) is, at first sight, confusing if not misleading; at a later stage (see p. 103), however, this difficulty will be shown to be more apparent than real. For a more detailed consideration of limbs acting as levers, see Appendix II, p. 113.

Interaction of adjacent limbs

A picture of the interaction between the limbs of a tetrapod can be obtained from Fig. 22, in which the two sides of the animal are assumed to be symmetrical in posture and vertical loading of the limbs; the angles of inclination of the front limbs (α) are not the same as those (β) of the hind limbs. In order that equilibrium should exist, the weight of the body must be distributed in accordance with the principles defined in § I, and since the loading is symmetrical on the two sides of the body the relative loads carried by the front and hind limbs must be inversely proportional to the distances of the feet from the centre of gravity of the body. In the absence of horizontal transverse forces, the longitudinal horizontal thrust applied to the body by one limb must be equal and opposite to that applied by its ipsilateral fellow. If the vertical loads on the right fore and right hind limbs are V_1 and V_4, the fore limb in its capacity as a strut exerts a posterior horizontal thrust to the body equal to $V_1 \tan\alpha$, where α is the angle of inclination of the limb's axis; similarly the hind limb exerts an anterior thrust equal to $V_4 \tan\beta$, where β is the angle of inclination of its axis. If the system is to be in equilibrium this inequality must be compensated by extrinsic muscular action; if (as in the figure) $V_4 \tan\beta > V_1 \tan\alpha$ this muscular action must be such as to apply a posterior longitudinal force to the body and posterior friction at the feet. Compensation of this type can be effected in a variety of ways.

(i) The protractor muscles of the front limb can exert a posterior thrust (H_{FP}) to the shoulder (and elicit posterior friction at the front foot) such that $H_{FP} = H_4 - H_1$; the hind limb functions solely as a strut (Fig. 22*a*).

(ii) The protractor muscles of the hind limb can reduce the anterior thrust exerted by this limb as a strut by developing a posterior force (H_{HP}) at the hip (and at the foot) such that $H_{HP} = H_4 - H_1$; the fore limb functions solely as a strut (Fig. 22*b*).

(iii) The protractor muscles of both limbs can co-operate in varying degrees so long as the horizontal forces which they exert are such that

$$H_{FP} + H_{HP} = H_4 - H_1.$$

In each of these three cases the total muscular effort is clearly the same.

(iv) Each limb can by its own extrinsic musculature reduce its horizontal thrust against the body and against the ground to zero. In this case the protractor muscles of the hind limb must exert a force (H_{HP}) equal and opposite to H_4, and the retractor muscles of the fore limb must exert a force (H_{FR}) equal but opposite to H_1 (Fig. 22*c*); again the net posterior muscular thrust is $H_4 - H_1$, although the total muscular effort is $H_4 + H_1$.

(v) The forward thrust of the hind limb can be increased by activity of its retractor muscles (Fig. 22*d*): in this case the effort required from the protractor muscles of the front limb must be correspondingly increased also.

At first sight it might appear that the states of equilibrium shown in Fig. 22*c* and *d* are wasteful of effort; it will be shown later that they are of considerable functional interest. For the moment, however, it should be noted that for any given position and loading of the feet the minimum muscular effort essential for equilibrium is always the same, but its distribution between the various muscles is under the control of the animal. Without altering the vertical loads being carried by the limbs and without altering the position of its feet an animal can transfer the strain from one muscle to another. Before considering further the functional significance of this conclusion, it is convenient to note that the range of muscular activity conformable with equilibrium can be still further widened by changing the distribution of the body weight between the four limbs. For example, if the vertical thrust of the right hind limb is suitably reduced and that of the right front limb increased, these two limbs can (as in Fig. 23*a*) act as struts, whose common centre of pressure (G_1) lies anteriorly to the line of action (G) of the body weight; equilibrium in respect to vertical forces can exist so long as the common centre of pressure (G_2) of the two left feet lies posteriorly to G and $GG_1 = GG_2$ (see Fig. 23*b*). This can be effected by adjusting the vertical thrusts of the two left limbs; if, now, the left front limb acts as a strut the friction acting at the foot will be $V_2 \tan \alpha$, whilst that of the left hind limb will be $V_3 \tan \beta$. If the right hind, right fore, and left fore feet are all acting as struts the left hind limb will be in equilibrium as long as its extrinsic muscles compensate the difference $V_3 \tan \beta - V_2 \tan \alpha$. The animal shown in Fig. 22 or 23 can therefore stand in equilibrium without strain of any of its extrinsic limb musculature apart from that of the left hind limb. In other words, by modifying the distribution of weight between the various limbs an animal can transfer strain on its extrinsic muscles from one side of the body to the other. In every case the total amount of muscular strain borne by the muscles is the same.

In the above examples it is assumed that the limbs do not exert horizontal transverse forces against the body. As already pointed out, however, a horizontal longitudinal couple applied to the body by the limbs can be compensated by a horizontal transverse couple due to the limbs acting as transverse levers under the action of adductor or abductor muscles. From all these facts it is obvious that for any given standing posture the strain which is placed on the musculature by the weight of the body can be distributed in a variety of ways and that, for this reason, the whole of the musculature must be regarded as a single functional unit and not as a series of separate muscles each playing a more or less independent role. This conclusion is probably not unrelated to the fact that many mammals can stand for long periods without fatigue. As will be shown elsewhere, the picture of the whole of the limb musculature as a single functional unit also applies to the animal in motion; as shown by Stewart (1938) very extensive muscular excision can be effected before the animal loses its normal locomotory gait.

Distribution of muscular effort in relation to function

From the above analysis it is clear that there is a wide but definable range of muscular patterns within which the mechanical requirements of any standing posture can be met. It is also clear that the actual strain imposed on the musculature of a joint could be defined if the magnitude and direction of the reactions of the ground against the feet were known: these values can only be obtained experimentally. Pending the availability of such data the following considerations are of some interest.

(i) *Limiting angle of friction.* If an animal is standing on ice little or no friction can operate at the feet, and no horizontal thrust can be transmitted from one limb to another by the vertebral column; each limb must be completely stabilized against the weight of the body by the activity of its own extrinsic musculature (Fig. 22*c*). In a less extreme case, the amount of friction required to stabilize a limb must not exceed that permitted by the limiting coefficient of friction between the foot and the ground—in other words, the inclination of the limb must not exceed a critical value before its extrinsic muscles come into play. If, however, the limb is able to engage the surface of the ground with claws or other devices there need be no such limit to the degree of protraction or retraction of the limbs (see, however, §§ IV and V).

(ii) *Equitable distribution of muscular strain.* On general grounds it may be assumed that an animal will not adopt a pattern of muscular activity which places an undue strain on any of the muscles concerned; in other words, it will tend to adopt a pattern which distributes the strain between the muscles in proportion to their ability to develop and sustain tension. For example, in Fig. 22*a* the whole strain falls on the protractors of the fore limbs and none on the retractors of the hind limbs. In Fig. 22*b* the situation is reversed, and, as already explained, the strain can be distributed between these two groups of muscles in any proportion. In other words, by voluntary contraction of one of these groups the other can be partially or completely relieved of all strain. On the other hand, as in Fig. 23, the strain can be distributed in various proportions between the two sides of the body. In the absence of observational data, it is impossible to say which particular pattern of muscular activity will be adopted, but it may be presumed that for any given posture of limbs the animal will adopt a pattern wherein the relative strain falling on each group of muscles will bear some relationship to its strength. Just as the moment of the reaction at the foot about the hip or shoulder joint is a measure of the strain placed on the muscles of these joints, so the strain on the other joints of the limb is also determined by the moment of the foot reaction about these joints. This aspect of limb stability will be considered elsewhere (see §§ IV and V). In general it may be said that the most 'comfortable' pattern of muscular activity, within the possible range of equilibrium, will probably be that in which the reaction from the foot passes closest to the limb joint whose extrinsic musculature is weakest.

The correlation of standing posture with an economical distribution of strain between the relevant muscles has already been noted in the case of an animal standing either freely or under restraint on an inclined surface (see p. 95).

Summary

1. The limbs of a tetrapod can contribute towards the support of the body either in the capacity of struts or in the combined capacity of struts and levers. When acting as a strut a limb exerts forces along its own mechanical axis only, the moment of the muscular tensions operating about the hip or shoulder joint being zero. When a limb is acting as a lever the limb exerts both against the body and against the ground forces at right angles to its mechanical axis; it is able to do so by means of the muscles whereby the limb is attached to the body.

2. When a limb is acting as an inclined strut the couple which is exerted on it by the weight of the body must be compensated by a couple due to (i) a horizontal force exerted on its proximal end by the body, and (ii) friction or other horizontal force acting at the foot. The horizontal force acting at the proximal end of the limb represents the resultant horizontal force exerted on the body by the three other limbs. Instances are described of two or more limbs co-operating, either as longitudinal or transverse struts, for the support of the body.

3. When subjected to tension from a muscle originating on the body, a limb operates as a lever and exerts equal but opposite horizontal forces against the ground and against the body. At the same time it exposes the body to a turning couple whose moment is equal to that of the muscle's tension about the centre of rotation of the joint. If the weight of the limb be neglected, the resultant of the horizontal force and couple applied to the body by the muscle is equal in direction and magnitude to the horizontal force exerted by the ground against the foot.

By means of the muscles which operate between the body and the limb an animal can control the horizontal forces exerted by a limb against the body and against the ground. The magnitude of the vertical force exerted by a limb against the body is almost entirely controlled by muscular effort operating along the mechanical axis of the limb.

4. The muscular co-ordination between two or more limbs, acting as levers, is described. For any given position of the feet relative to the centre of gravity of the body, the minimum total muscular effort required to maintain equilibrium is always the same. The distribution of this effort between the muscles of the various limbs can, however, be varied between relatively wide limits, either by changing the tension of one or more extrinsic muscles or by changing the distribution of weight between the feet by a change in the axial thrust of the limb. The whole of the limb musculature of the animal must be regarded collectively as one functional unit.

5. The particular pattern of muscular activity adopted for any given posture and loading of the limbs is probably such that the strain is distributed between the relevant muscles in proportion to their ability to develop and sustain tension.

IV. THE EQUILIBRIUM OF INTRINSIC LIMB JOINTS

The arguments developed in the previous section are based on the assumption that each limb can be regarded as a telescopic structure capable of exerting a longitudinal axial thrust and of acting as a lever when operated by the muscles by which the limb is attached to the body. The tetrapod limb is, however, essentially a series of articulated rods whose joints possess little or no natural rigidity and whose ability to resist bending forces depends almost entirely on the activity of their associated muscles. It is therefore necessary to consider the type of internal muscular effort which enables the limb as a whole to function as an efficient strut or lever.

From a mechanical point of view the whole limb can be regarded as a series of hinged rods in equilibrium with three groups of external forces: (i) the forces exerted on the limb by the body, (ii) the force exerted by the ground against the foot, (iii) the weights of the individual limb segments. Since the mechanism whereby rigidity of the limb is preserved in respect to the weights of the limb segments is essentially the same as that in respect to the forces exerted on the limb by the body (see p. 105) it is possible to simplify the system and consider the limb, at rest, as in equilibrium with two equal but opposite external forces, one applied by the body and the other by the ground. Three typical instances are shown in Fig. 24*a–c* in which the posture of the various joints is the same in each case and where the joint A is vertically beneath joint H. In Fig. 24*a* the only force (W) exerted by the body on the limb is the weight of the body acting vertically through the centre of rotation of joint H; the centre of pressure of the foot lies vertically beneath joint A. The knee (K) is the only joint about which either the weight of the body or the reaction (W_1) of the ground exerts a turning moment, and flexion of this joint can be prevented by adequate tension in the muscle (M_1) operating about this joint; the tension of the muscle must be such that its moment about K is $W.Ka$. The whole limb is operating as a vertical strut.

In Fig. 24*b* the action (R) of the body on the limb represents not only a fraction of the body weight acting at K, but also an anterior horizontal pull (P) by the pelvis against the head of the femur. If the limb is to support the body it must do so as an inclined strut whose axis is HC, where C is a point on the plantar surface at which the line of action of the force (R) exerted by the body meets the ground. Internal stability of the limb depends on the development by the extensor muscle of the knee of a moment equal to $R.Ka_1$, and also on a moment ($R.Ab$) exerted by the extensor muscle (M_6) about the ankle joint (A). Provided that the relative tensions of the two muscles are such that the ratio of their moments about their respective joints is always Ka_1/Ab, the whole limb will continue to act as an inclined strut whose axis is HC, and whose axial thrust along this line will vary with the absolute value of the tensions developed by the muscles M_1 and M_6. On the other hand, any change in the relative tensions of the two muscles will cause a movement of the centre of pressure of the foot against the ground and therefore will change the axis of the limb; for example, if the tension of M_6 becomes zero, the centre of pressure moves to a point vertically below the joint A.

In Fig. 24*c* the force (R) exerted on the limb by the body does not pass through joint H, and therefore exerts a moment about joint H. Stability of the limb depends, as shown in § III, on the muscle M_5 developing a moment about H equal but opposite to $R.Hc$; at the same time M_1 must develop a moment $R.Ka_2$ and muscle M_6 a moment $R.Ab_1$. In short, the internal stability of the limb is secured so long as the tension of the muscles operating about the several joints are related to each other in direct proportion to the distances of the joints from the line of action of the force exerted on the limb by the body—or, in other words, from the line of action of the reaction of the ground against the foot.

In § III the whole musculature of the limb has been regarded as though it were divided into two separate categories, one enabling the limb to exert forces along the mechanical axis of the limb and the other exert horizontal forces at the feet. This classification is, of course, entirely empirical, and it is necessary to consider its relationship to the morphology of the muscles as distributed in life. So long as the limb is acting as a strut as in Fig. 24*b*, it is legitimate to regard the muscles operating about the joints A and K as axial muscles enabling the limb to exert forces along its mechanical axis only, and as carrying the whole of the body weight which rests on the limb. On the other hand, if the limb is exerting a horizontal force (F)—apart from that due to the action of the limb as a strut—against the ground as in Fig. 24*c*, it can only do so if the muscle M_5 operating about joint H is active; in this sense, muscle M_5 can be regarded as an extrinsic muscle capable of operating the limb as a lever. It is obvious, however, that the activity of muscle M_5 cannot enable the foot to exert a force (H) against the ground unless muscles M_1 and M_6 co-operate by an adjustment of their tensions such as will compensate the turning moment of the force H about the knee and ankle. In this respect the muscles M_1 and M_6 form an essential part of the mechanism whereby the limb as a whole operates as a lever. The mechanism whereby the limb as a whole is operated as a strut and as a lever is further illustrated by Fig. 24*d*–*f*. In Fig. 24*d* the limb is resisting a reaction (R) from the ground and is stable so long as the muscles M_8, M_1 and M_6 exert adequate turning moments about their respective joints. The reaction (R) can, however, be resolved into an axial thrust (T) and into a horizontal frictional force (F) acting at the foot; the limb is stabilized in respect to the axial force (T) by means of the muscles M_1 and M_6 (Fig. 24*e*) and in respect to the force (F) by the muscles M_8, M_1 and M_2 (Fig. 24*f*). Fig. 24*d* represents the resultant of Figs. 24 *e* and *f*. Effective control of the limb in its capacities as strut and lever depends, therefore, on the co-operative effort of the whole of its musculature, and this is probably dependent on a widespread pattern of myotactic reflexes.

Just as the force exerted on a limb by the ground can be resolved into components operating the limb as a strut or a lever, so it can be resolved into vertical or horizontal components. As shown in § II, the vertical load (V) on any limb is, within certain limits, under the control of the animal and that the horizontal (H) force acting at the foot is under similar control. For any given position of a joint relative to the centre of pressure of the foot, the bending moment about the joint is $Vx \pm Hy$, where x and y are the co-ordinates of the joint relative to the centre of pressure of the foot. Since both V and H are under control, the strain imposed on any particular joint is also under control, and this power clearly depends on the ability of the animal to adjust the relative and absolute tensions developed by all the muscles of the limb. In order that the limb should function efficiently, the whole of its musculature must act as a single functional unit in which the activity of each individual group of muscles is very closely co-ordinated with that of all the others. Doubtless, to a large extent, co-ordination is dependent on reflex myotactic phenomena, since any tendency for a joint to move under mechanical forces would stretch muscles capable of applying compensating moments about the joint.

The far-reaching co-ordination between the muscles of a pair of ipsilateral limbs can be visualized partially from the example illustrated in Fig. 25. In this particular case the distribution of weight between the front and hind limbs is assumed to be in the proportion of 4 : 5; by control of the protractor or retractor muscles of either limb the animal can vary the amount of friction acting at the foot but (so long as the transverse extrinsic muscles are inactive) an equal amount of friction must act at the other foot if the system is to be in equilibrium. Knowing the position of each limb joint and the magnitudes and directions of both foot reactions, the bending moments in respect to each joint can be calculated. This has been done in Table 1. It may be recalled that if the animal is standing when the longitudinal friction is not equal and opposite for an ipsilateral pair of limbs the resultant couple must be compensated by a definite degree of activity in the adductor or abductor extrinsic muscles. In all cases it is clear that there is a very widespread co-ordination between the whole of the limb musculature when an animal is in a standing posture although the animal can vary or even abolish the strain falling on any particular muscle. The requisite co-ordination is thus essentially of a dynamic nature and not such that each muscle must contribute a fixed unalterable effort.

The co-ordination between the muscles of each of four limbs can be illustrated by Fig. 26*a*. In this example, each of the four limbs is assumed to be acting as a vertical strut, the posture on the two sides of the body is the same, and the total body weight is distributed equally between the four feet. The posture of the limbs is such that the centre of pressure of each fore limb lies vertically under the centre of rotation of the wrist (B), while that of each hind limb lies posteriorly to the ankle (A). No strain falls on the muscles operating about the hip joint, but the knee must resist a flexor moment ($W_{BH}Ka$) by activity of its extensor muscles (M_1). Similarly, in the fore limb the humero-scapular joint must resist a flexor moment ($W_{BF}Gb$) and the elbow a flexor moment ($W_{BF}Ee$). The dynamic co-ordination between the muscles of both limbs can be visualized by imagining the extensor muscles (M_3 and M_4) of the fore limb to reduce their tension to two-thirds of their original value (Fig. 30*b*), without any change occurring in the two right limbs. In order to maintain equilibrium, the left hind limb must exert an increased vertical thrust against the body, and it must act through a point (C_1) on the plantar surface such that its moment about G_2 is equal to that of the thrust of the fore limb. This condition is fulfilled by (i) development of suitable tension in the retractor muscles (M_5) of the limb; (ii) an ability of the ankle to resist, by means of its extensor muscles (M_6), a moment ($W_{BH}.C_1A$) and of the joint P to resist an extensor moment ($W_{BH}.C_1P$) by its flexor tendons or muscles (M_7); (iii) a reduction of the strain on the extensor muscles (M_1) of the knee to ($W_{BH}.Ka_1$). Alternatively, a variation in the distribution of the body weight between individual limbs (within the limits defined in § II) enables a tetrapod to increase or decrease the strain falling on the musculature of any of its intrinsic limb joints.

Table 1

The horizontal columns of the table show the calculated moments of the muscles of the right side of the animal shown in Fig. 25 for different values of friction acting inwards at the feet. Total weight of body 18 lb.; load on right fore limb 4 lb., load on right hind limb 5 lb.

Friction	Muscular moments—in arbitrary units											
	Shoulder		Glenoid		Elbow		Hip		Knee		Heel	
	Prot.	Retr.	Ext.	Flex.	Ext.	Flex.	Prot.	Retr.	Ext.	Flex.	Ext.	Flex.
0	—	4	—	1·2	5·2	—	10	—	11·25	—	0·75	—
0·2	—	3	—	0·54	4·7	—	9	—	10·65	—	1·05	—
0·3	—	2·5	—	0·21	4·45	—	8·5	—	10·35	—	1·20	—
0·4	—	2	0·12	—	4·2	—	8	—	10·05	—	1·35	—
0·6	—	1	0·78	—	3·7	—	7	—	9·45	—	1·65	—
0·8	—	0	1·44	—	3·2	—	6	—	8·85	—	1·95	—
1·0	1	—	2·1	—	2·7	—	5	—	8·25	—	2·25	—
2·0	6	—	5·4	—	0·2	—	0	—	5·25	—	3·75	—
3·0	11	—	8·7	—	—	2·3	—	5	2·25	—	5·25	—
4·0	16	—	11·0	—	—	4·8	—	10	—	0·75	6·75	—

The digitigrade habit

In Fig. 26*b* three limb joints (K, A and P) lie between the hip and the point of application of the reaction of the ground against the foot, and in such cases the animal can change the position of the ankle and knee without altering the mechanical equilibrium of the limb as a whole. By flexing the knee and extending the ankle (Fig. 27), the latter joint can be moved closer to the line of action of the body weight and ground reaction thereby reducing the strain on its extensor muscles to $W_{BH}A_1a_2$, but placing an increased strain ($W_{BH}K_1k_2$) on the extensors of the knee. In plantigrade limbs this principle operates particularly when the forward position of the centre of pressure of the foot is due to the limb being in a retracted posture (Fig. 28) either when the animal is walking or standing with a limb retracted. If the foot remained plantigrade the strain on the ankle would increase linearly with the degree of retraction of the limb. In practice, the maximum strain on the ankle joint is determined by the position of the metatarso-digital joint (P), for if the limb is retracted far enough to cause the centre of pressure of the foot to pass through the centre of rotation of this joint, the strain on the extensors of the ankle can be relieved by raising the knee and extending the ankle joint.

The application of this principle to all four limbs suggests that the digitigrade habit characteristic of cursorial mammals is to be associated with the normal posture of the feet relative to the points at which the weight of the body is transferred to the hips and shoulder joints. In limbs of this type the metatarso-digital and metacarpo-digital joints lie much farther back relative to hips and shoulders than in plantigrade forms. As shown in Fig. 28, the flexure of the knee, whilst relieving the strain on the extensors of the ankle, involves a greater strain on the knee joint itself; and similarly, raising the elbow to relieve the wrist places an additional strain on the extensors of the elbow. By extending the knees and elbows and simultaneously protracting the humerus and retracting the femur, the hips and shoulders are raised, thus relieving the strain on the extensors of knees and elbows and (see Fig. 29) yielding the typical digitigrade posture.

The ankle cannot be raised until the centre of pressure has passed to the distal end of the metatarsal bones and the effort required to extend the joint against the weight of the body increases with the length of these bones. In a plantigrade form there must be a correlation between the length of the bones and the strength of the extensor muscles of the ankle. Once the latter joint is raised, however, as in a digitigrade form, the length of the metatarsals can be greatly increased before a limit is reached at which undue effort is required to extend the joint against the weight carried by the foot. Thus in Fig. 29*d* the metatarsals can be four times as long as in Fig. 29*b* before the same effort is required to extend the ankle. It seems likely that the elongated bones of digitigrade mammals with consequent increase in the length of the stride is a natural sequel to the elevated posture of the ankle and wrist joints. Just as longitudinal movements of the centre of gravity of the body tend to displace the centre of pressure of a plantigrade limb between the heel and toes, so lateral movements tend to displace it laterally on the plantar surface. Animals whose bodies are relatively free from lateral rolling movements may be expected to dispense with lateral digits. It would be of interest to consider the evolution of the tetrapod foot from this point of view.

Curvilinear muscles and muscles or ligaments common to more than one joint

Throughout the whole of the previous discussion the action of a muscle between its points of origin and attachment has been assumed to be one of rectilinear tension (see Fig. 30*a*). While this is true for certain muscles of the tetrapod limb, it is by no means true for all; in some cases the muscle changes its direction between its points of origin and attachment and therefore when under tension it exerts a pressure on underlying structures, including the joint about which the muscle operates; not infrequently, a sesamoid bone may be present in the neighbourhood of the joint. None of these considerations affect the general conclusion that the final result of muscular activity is the development of a turning moment at the joint proportional to the distance of the line of action of the muscle from the centre of rotation of the joint.

In Fig. 30*b*, a muscle (M) is attached to a bone (A) at an effective centre of origin (a); the muscle passes smoothly over the head of the bone to its site of attachment on the bone B at O; the tension of the muscle is such that the joint is in equilibrium with the external forces F_1 and F_2 which are equal and opposite to each other. In order to preserve equilibrium of bone B, the tension (T_1) of the muscle acting at O must (if the joint be frictionless) be such that the resultant (R_1) of T_1 and F_1 passes through the centre of rotation (p) of the joint, and the force (R_1) must be at right angles to the surface of the joint. The forces exerted by the muscle against the bone A are, however, (i) its tension acting at a and (ii) a pressure exerted by the surface of the muscle against the bone between x and y. If the joint is in equilibrium (and free from friction) this pressure acts through the centre of rotation of the joint and is equal and opposite to the resultant of R_1 and R_2; the net effect of the muscle on bone A is the resultant of P and T_2; this is equal and opposite to the force T_1. The system is essentially unchanged if the pressure of the muscle is exerted against both bones with or without the interposition of a sesamoid bone (Fig. 31*b*) whose essential functions are to increase the working distance of the muscle from the centre of rotation of the joint and provide a bearing surface for the main bones forming the joint.

In a large number of instances curvilinear muscles traverse more than one joint, and so long as the external bending forces pass on the same side of these joints they can all be partially or completely stabilized by tension in a single long muscle. A simple analogous system is shown in Fig. 32. Complete stabilization of successive joints by means of one long muscle is possible if the distances of the joints from the line of action of the external bending forces bear to the working distances of the muscle from the joints a ratio which is the same in all cases. For any other condition one or more 'short' muscles (one-joint muscles) must be operative. Examples of muscles organized into 'short' and 'long' elements are frequent in all tetrapod limbs.

The effect of the limb weight on the stability of intrinsic limb joints

As in the case of the whole limb, a complete picture of the muscular co-ordination necessary for the maintenance of intrinsic limb stability involves a consideration of the strains imposed on the joints by the weights of the individual limb segments. The effect of these forces is, however, of the same fundamental nature as that of the weight of the body. For any given posture a force equal to the weight of the whole limb will act at a centre of pressure on the foot and exert a moment about each of the intrinsic limb joints. The total strain imposed by gravity on the musculature of a joint will therefore represent the sum of the moments of the two forces, (i) the total vertical force exerted by the ground against the foot, (ii) the weight of the limb segments lying distally to the joint.

SUMMARY

1. The patterns of muscular co-ordination necessary to maintain the functions of the whole limb as a strut or lever are described. When functioning solely as a strut, the stability of the limb depends on the co-ordination of the muscles acting about each joint situated distally to the hip or shoulder but proximally to the centre of pressure of the foot; when the limb is functioning as a lever the above muscles must also be co-ordinated with those operating about the hip or shoulder.

2. The quantitative muscular effort required for the stabilization of any limb joint depends on the magnitude and direction of the reaction of the ground against the foot and on the distance of the centre of rotation of the joint from the line of action of this force. Since the reaction of the ground against the foot is under the control of the animal, the latter can continue to stand whilst varying the strain falling on any one intrinsic or extrinsic muscle provided it readjusts the activity of all the others not only in the same but also in all the other limbs. Instances are given of the co-ordination of the musculature in the four limbs of an animal standing at rest.

3. The digitigrade habit is associated with a posture of limbs in which the line of action of the body weight passes anteriorly to the distal metacarpal or metatarsal joints.

4. The forces exerted on joints by curvilinear muscles are discussed. In general the centripetal pressure exerted by the muscle is equal and opposite to the resultant longitudinal thrusts exerted by the two main bones composing the joint. Brief reference is made to multi-joint muscles.

5. The stabilization of intrinsic limb joints to the weight of the limb itself is of the same fundamental nature as that to the weight of the body.

V. THE EQUILIBRIUM OF THE VERTEBRAL COLUMN

Before considering the general effect of the weight of the body on the stability of the vertebral column it is necessary to bear in mind that the column represents a highly complicated anatomical system. Each joint is usually of considerable complexity, and the distribution of the vertebral muscles is by no means simple. To some extent the form of the intervertebral articulations show fairly clear and definite relationships to the degree and type of movements which the animal normally exercises about the joint, but, so far as is known, this point has yet to be examined systematically. Further, it is by no means easy to determine with any accuracy the precise distribution and function of the various components of the vertebral muscles. Many of the observations necessary for an adequate functional study have therefore yet to be made, although the illuminating approach to the mechanics of the problem made by D'Arcy Thompson (1942) makes further analysis highly desirable. In the meantime, the strain imposed by the weight of the body on the muscles and ligaments of the vertebral column can be examined by methods essentially similar to those already applied to the joints of the limb, and the results obtained can be regarded as an indication of the lines which might be followed for future experimental treatment.

At the outset it is useful to note that the external forces operating about each intervertebral joint may produce one or more of six types of distortion in the vertebral column: (i) longitudinal bending in a vertical plane thus tending to arch (flex) or sag (extend) the back, (ii) transverse bending in a horizontal plane, tending to curve the body to the left or right side, (iii) torsion about a longitudinal horizontal axis rotating one vertebra on its neighbour, (iv) vertical shear, (v) transverse shear, and (vi) longitudinal compression or tension of the vertebral axis. Of these six types of distortion the last three are resisted passively by the inherent properties of the bones, intervertebral facets, and similar structures, and the power of the vertebral column to resist such distortion does not change markedly when all muscular activity is eliminated. In the case of bending and torsional stresses, however, only gross distortion is compensated by ligaments or other passive structures; during life effective control is exercised by muscles.

To provide a picture of the forces which must act across an intervertebral joint in order to maintain the equilibrium of the vertebral column at this level, it is necessary to consider the relationship of the joint to all external forces whatever be the nature of the distortion which they tend to produce. For other purposes, however, it is more useful to consider the variation in the magnitude of one particular type of distortion at different joints in the column.

Longitudinal bending

Longitudinal bending in a vertical longitudinal plane passing through the centre of rotation of all the vertebral joints is induced by (*a*) all vertical forces acting on the column (i.e. weight of the body, vertical thrusts of the limbs, and vertical components of all muscles under tension), (*b*) all longitudinal horizontal forces acting dorsally or ventrally to the neutral axis of the column. Two simple cases of vertebral equilibrium are illustrated in Fig. 33. In this figure each of the four limbs is assumed to be acting as a vertical strut, and the two sides of the body are assumed to be functionally symmetrical in the sense that the loads of the left fore and left hind feet are equal to those of the right fore and right hind feet respectively. The vertebral column is therefore free from torsional stresses, and the plane of action of all the vertical and muscular forces acting on the body can be regarded as coincident with the vertical median plane of symmetry of the body. So far as a cervical joint (V_1) is concerned, the conditions of equilibrium are determined by the weight of the head and neck. If the centre of gravity of all the structures lying anteriorly to V_1 be at G_1 and their weight be W_{HN} the bending moment about V_1 is $W_{HN}x_1$. In order to prevent the head and neck sagging downwards the dorsal musculature (M_1) of the neck must develop a tension (T_1) about V_1 such that $T_1 z = W_{HN} x_1$; under these conditions the resultant thrust (R_1) of the body against the joint must be equal and opposite to the resultant of T_1 and W_{HN}. The mechanism is essentially that of a crane. As pointed out by D'Arcy Thompson, animals with heavy heads or long necks are able to exercise a high moment by their neck muscles owing to the presence of long thoracic spines for the attachment of the muscles. When the system is in equilibrium, the head and neck are subjected to two equal and opposite couples, one due to their own weight and the upward thrust of the body at V_1 and the other to the tension of the muscles and the corresponding reaction at V_1. Further, the total reaction (R_1) at the joint has a vertical shearing component (*Sh*) and a longitudinal compressional component (C_1); these forces must be resisted by the intervertebral facets.

In the case of a lumbar vertebra (V_2) the conditions are essentially similar except that it is necessary to consider the moment due to the forces exerted by the limb against the pelvis. In the case of the animal shown in Fig. 33, the upward moment of the force (P) acting on the pelvis about the joint V_2 is greater than that of the downward force (W_{HQ}) acting through the centre of gravity at G_3, and consequently the joint must be stabilized by tension (T_2) in the abdominal or psoas muscles (M_2) acting ventrally to the joint

$$T_2 z_2 = P\,(x_2 + x_3) - W_{HQ} x_2.$$

The reaction (R_2) acting at the joint can be determined from the triangle *abc*, where *ab* represents the resultant of the forces P and W_{HQ}; in this particular case the compressional component of R_2 is much greater than its shearing component.

If a limb exerts a horizontal force against a hip joint which lies ventrally to the vertebral axis its effect will be to reduce or increase the moment of the total reaction at this point about the joint V_2. If the horizontal thrust of the limb against the pelvis is directed anteriorly, the moment of the hip reaction about V_2 will be reduced; if the thrust against the pelvis acts posteriorly the moment of the hip reaction about V_2 is increased. Thus by exerting the retractor muscles of the hind limb an animal can relieve the strain imposed by the weight of the body on its ventral musculature (M_2). As explained in § III, the hind limbs of an animal at rest can only exert a resultant anterior horizontal force against the body, if the fore limbs exert a corresponding posterior thrust; in other words, the tendency of the back to sag downwards under the weight of the body or of a superimposed load can be reduced by bracing the limbs outwards against the ground. This mechanism is brought into action when a horse or mule is standing with a heavy load on its back. It is illustrated in Fig. 34 (from Rademaker, 1931). Similarly, if, with all feet extended outwards from the centre of the body, an animal maintains (by means of front retractor and hind protractor muscles) a vertical reaction at each of its feet, the strain on the ventral musculature of the body is correspondingly increased. Thus when standing on a smooth surface the extent to which the limbs can be extended depends not on the strength of the limb muscles but on that of those ventral muscles required to maintain the rigidity of the back (see Fig. 35).

Distribution of strain along the vertebral column

A more generalized picture of the longitudinal bending strain imposed by the weight of the body on the muscles of the vertebral column can be obtained from a consideration of the bending moment curve for the whole of the column. Starting with an elongated type such as that shown in Fig. 36, the column can be regarded as a series of rigid vertebral segments loaded midway between each intervertebral hinge in accordance with the figure shown at each arrow. Assuming all the limbs to act as vertical struts and the feet on the two sides of the body to be symmetrically loaded, the load resting on the front and hind feet can be determined. Bending moments taken about each of the fourteen hinges shown in the figure yields the bending moment curve indicated by the dotted line; moments tending to make the dorsal side of the body convex are shown above the base line, and those tending to make it concave are shown below the base-line. It will be noted that the sites of maximum strain on the dorsal muscles lie over the hips and shoulders, while the maximum strain on the ventral muscles lies approximately midway between shoulders and hips.

The whole bending moment curve represents (Fig. 37) the sum of three separate curves: (i) that due to the head and neck *ABC*, (ii) that due to the tail *DEF*, and (iii) that due to the region (*DGHIC*) of the body lying between the shoulders and hips *DGHIC*. The form of the whole curve therefore depends on the relative lengths and weights of the head and neck, trunk and tail. The effect of this principle is shown in Fig. 38. The animal in Fig. 38*a* represents the animal in Fig. 36 after the removal of the three central segments of the body; the head, neck and tail are now sufficiently large to remove all strain from the ventral musculature. In Fig. 38*b*, however, the tail has been removed and the strain thereby placed on the ventral muscles which, although considerable, is very much less than that in Fig. 36. In every case the site of maximum strain on the ventral muscles (i.e. the point of inflexion of the bending moment curve) is the site at which no shearing forces are present.

In Fig. 38*c* the distribution of the weight between joints 3 and 6 has been slightly modified from that shown in Fig. 38*a*. In Fig. 38*c* the bending curve becomes tangential to the base-line, in other words at joint 4 there is no bending moment and no shearing forces, and since no compression forces are acting along the column it is possible to make an imaginary vertical section through this joint without disturbing the equilibrium of the system. This particular condition is essentially that with which the general tetrapod system has been compared by D'Arcy Thompson (1942), and it represents the animal as two balanced cantilevers. It is, however, difficult to avoid the conclusion that such a form is really a very specialized (and possibly quite theoretical) case of a much more generalized proposition. As pointed out by Thompson, it is difficult to see how animals with small heads and long bodies can be regarded as a pair of balanced cantilevers; they can, however, readily be harmonized with the generalized type shown in Fig. 36.

As already mentioned, an animal is able to control the form of its bending moment curve by exercising leverage against the ground with its limbs. The general nature of this control is illustrated in Fig. 39. As explained in § III, an extrinsic muscle operating between the body and a limb exercises on the body a couple which is equal and opposite to that exercised on the limb. Any such couple can be resolved into compression components along the column and a couple acting at right angles to the column. Thus if the limbs are braced outwards by the protractor muscles of the fore limbs and the retractor muscles of the hind limbs, the effect is to apply a pair of couples (C_1, C_2; C_3, C_4) as shown in Fig. 39; the bending moment of either of these couples about each vertebral joint is such as to render the back convex and thus relieve the ventral musculature. How far the natural form of the vertebral column is to be correlated with the strains imposed on it by the weight of the body (see D'Arcy Thompson) remains a fascinating subject for study, but it must be borne in mind that the effective loading of the column is definitely under control of the animal by means of its muscles—and that the greatest strains are probably those imposed by the muscles when the animal is in locomotion. It is, however, clear that if an animal is to stand when using its legs as vertical struts there must be a definite correlation between the weight and length of the body between the points of support with (*a*) size of head and tail and with (*b*) the strength of the abdominal and allied musculature.

Lateral bending

Any horizontal force acting on the body will tend to cause lateral bending at a vertebral joint if its line of action does not pass through the centre of rotation of the joint. The whole of the external horizontal forces acting on the body are due to the activity of the limbs in their capacity as struts or as levers, and so long as the animal is at rest the resultant of all these forces represents a pair of equal and opposite forces. If the line of action of these two resultant forces lies in the vertical plane occupied by the centres of rotation of the thoracic and lumbar vertebrae no lateral distortion is involved; under all other circumstances bending will occur to one side or the other unless restraint is imposed by the lateral musculature of the column. A simple instance of equilibrium in respect to lateral bending forces is shown in Fig. 40, where the unequal compression exerted by the limbs on the two sides of the body is compensated by tension in the vertebral musculature on the right side of the body. In the case of reptiles and urodeles transverse horizontal forces are probably of greater importance than in the case of mammals. Fig. 41*b* indicates a condition in which the horizontal forces exerted by the limbs on the body will tend to bend the latter into an S-shaped curve unless restrained by appropriate tensions in the lateral musculature.

It should be noted that just as forces exerted by the limbs against the ground exert a strain on the lateral musculature of the back, so intrinsic activity of the back musculature can induce a reaction by the limb against the ground (see p. 93).

Longitudinal torsion

If, in the case of a mammal, the vertical thrust exerted against the body by any one limb is equal to that of its contralateral fellow, the vertebral column will be free from torsion; if the two thrusts are not equal, the consequent torsion must be compensated by muscular or ligamentous torsion. It may be noted that for any given position of the feet and centre of gravity of the body there is only one distribution of the weight between the four limbs which is consistent with an absence of torsional strain (see § II, p. 93). Further, in so far as torsional forces can be initiated by intrinsic action on the part of the vertebral musculature, so an animal can thereby control the amount of weight falling on any one of its feet. It seems likely, however, that torsional effects are most obvious when an animal is in rapid motion, and are largely compensated by the action of ligaments rather than of muscles.

SUMMARY

1. The nature of the strains imposed on the vertebral column by the weight of the body and by the forces exerted by the ground against the feet of a tetrapod are discussed.

2. The mechanical picture presented by a tetrapod is essentially that of a flexible overhung beam supported by four elastic legs; only under very special circumstances can the body be compared to two balanced cantilevers.

3. By means of the muscles of its limbs an animal can increase or decrease the strain falling on its vertebral musculature or vice versa.

noon wore on, he kept that isolated and puzzled detachment of the Austrian power inactive, until at ten at night they were induced to lay down their arms, several hours after their superiors had capitulated on their behalf.

It was probably while Manin was absent on his tour of inspection in the heart of the Arsenal, that the mutiny of the Italian-speaking troops took place in front of the Lion Gate. Various bodies of white-coats were being hurried up to secure the Arsenal, first the marines and then a detachment of the Wimpffen regiment, in each case to find the ground outside the gate occupied by a resolute body of Civic Guard, who denied them entrance. On both occasions the Italian soldiers when ordered to fire, disarmed and arrested their foreign officers; the Major of Marines showed fight and was wounded, but no one was killed. The mutineers plucked from their own uniforms the yellow and black badges of Austrian service and threw them by hundreds into the canal. Entering the Arsenal to complete the revolution which they had been sent to suppress, many of them hastened to strengthen the frail guard at the grill, behind which the Croats still lay crouching for a spring.

Manin on his return to the gate found himself master of the situation and able to speak to Martini with the voice of command. When the great bell of the Arsenal was rung to call the workmen together, an immense concourse of Arsenalotti, Civic Guards and revolted troops thronged all the spaces round the gate and swarmed in the rooms of the office above. Manin ordered Martini to give up the keys of the armoury where the muskets and swords were kept,[1] and on his refusal gave him five minutes, after which the door would be broken in. Ladders had already been brought and men were swarming into the armoury by the

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[1] The armoury was the room which is now (1923) the museum of the Arsenal; it is on a higher level than Martini's office and just round the angle of the yard. Martini's office was the building over the gateway which is still occupied by the Admiral of the Port, but the interior has been repartitioned and rearranged since 1848.

The capture of the Arsenal was only one part of the plan arranged on the previous night between Manin and his fellow-conspirators. The other part, the change of guard on the Piazza of S. Mark, was carried out by his agent Radaelli so smoothly and quietly that its importance has escaped the notice of many historians. Four cannon were standing loaded in front of S. Mark's door, capable, if necessary, of clearing the Piazza and keeping the crowd from again approahing the Governor's windows. This, the most important battery in Venice that day, was guarded by a company of Grenadiers—but they were Italians! Radaelli, with a body of Civic Guard fantastically armed, presented himself to the Austrian Captain and said that he had come to take his place. The Austrian laid his hand on his sword, but he was overpowered by his own men who sent him away free, plucked off their yellow and black badges, and joined themselves to the Civic Guard. By two o'clock Radaelli had sent word to Palffy that the cannon were pointed no longer at the people but at his windows. Owing to this *coup* it was safe and easy for another body of Civic Guard under Olivo and Bedolo to occupy the Governor's door and staircase.[1]

Palffy and Zichy had allowed themselves to be trapped by the Italians. If not already prisoners, they were cut off from all means of communicating with the Croats and the other loyal troops in the city. In such circumstances, with the Arsenal and fleet won over, the Italians could not find it difficult to extort a capitulation from these two men, never anxious to fight, who had now lost the means of fighting. The task was taken in hand by Mengaldo and the Municipality, who had refused to assist Manin, but who acted with vigour and initiative on their own account.

Mengaldo cannot be said to have exercised much control, as Commanding Officer, over the acts of the Civic Guard that day, and it was as well that he did not,

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[1] *Radaelli*, pp. 48, 50-53. *Marchesi*, pp. 116-117.

up. A crowd of Civic Guard, mutinied troops and newly armed Arsenalotti were waiting to follow him to S. Mark's. Taking in his hand an immense tricolor flag with the red cap of the Republic on the staff, he returned by the open route along the Riva degli Schiavoni which he had avoided on the way out. The Austrian guards stationed along the quay-side dared now offer no resistance, but turned out and saluted as the procession passed.[1]

The Piazza was thronged with a mob in the highest state of excitement and expectation, while at every window above were seen the faces of officials and of well-to-do citizens and their wives, anxiously watching the course of events. Mounted on a table, Manin with the banner in one hand and a drawn sword in the other proclaimed the Republic in the following words :—

> 'We are free, and we have a double right to boast of it because we have become free without shedding a drop of blood, either our own or our brothers', for I call all men brothers. But it is not enough to have overthrown the old Government; we must put another in its place. The right one, I think, is the Republic. It will remind us of our past glories improved by modern liberties. We do not thereby mean to separate ourselves from our Italian brothers. Rather we will form one of those centres which must bring about the gradual fusion of Italy into one. *Viva la repubblica! Viva la Liberta! Viva San Marco!*'

Some of the faces at the windows, Austrian and Italian, seemed puzzled or alarmed. But the populace below was frantic with delight, and after a moment's hesitation the Civic Guard joined the Republican movement by forming themselves into a square round Manin.

All Venice sprang to life and joy. Men ran down every alley shouting that the Republic was restored. The gondoliers rowed and raced like madmen to the farthest

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[1] *P. de la F.*, i. pp. 117, 139. *E. and F.*, pp. 344, 365. *Rovani*, p. 36. *La Forge*, i. pp. 272-273. He struck into the Riva at S. Sepolcro near San Giovanni in Bragora.

signed the capitulation.[1] The forts intact, the fleet, the material of war of every sort and the Italian-speaking troops were to be left behind 'in the hands of the Provisional Government which is going to be formed.' The foreign troops, between 3000 and 4000 strong, were to be shipped to Trieste, apparently without their arms, though this point was not made clear.[2] Events were to show that Palffy and Zichy might have stood out for the retention by the soldiers of their muskets. But otherwise it is hard to blame them for consenting to capitulate after they had lost the Arsenal, the fleet and the Piazza, and were themselves hostages in the hands of the insurgents, when half their troops had gone over to the Revolution and the Croats were isolated and surrounded in their barracks. But it may fairly be asked why they had allowed themselves to be manœuvred into that position by Manin.

The capitulation having been signed, the next business was the formation of a Provisional Government to carry out its terms. The Italian signatories of the capitulation—the Mayor, Mengaldo and five members of the Municipal Council—proceeded to form themselves into a Provisional Government, at any rate for the next few hours. They issued that same evening (22nd) a broadsheet announcing the capitulation and adding :—

> 'A Provisional Government will be instituted, and meanwhile, to meet the necessities of the moment, we signatories have been obliged to assume its powers on the spot. *Viva Venezia! Viva l'Italia!*'[3]

The word Republic and the word Manin were conspicuously absent from this proclamation, which fell

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[1] *M.C.V.*, *Cavedalis MSS.*, i. p. 116, gives the hour 6.30 and so do almost all other authorities. The capitulation is printed in *Errera*, pp. 435-436. It is signed by Zichy alone for Austria ; for the Municipality by Correr, Michiel, Medin, Fabris, Avesani, and Pincherle, and by Mengaldo for the Civic Guard.

[2] The capitulations signed for the Polesine, Friuli and Treviso on the following day on the model of Zichy's capitulation for Venice (see next chapter) were interpreted as allowing the foreign troops to depart with their arms in their hands.

[3] *Fogli Volanti*, iii. No. 4.

his colleagues to act with them for this purpose, on the ground that he was too ill and tired to leave his bed that night.[1] No doubt they had invited him to join them, and no doubt he had been too much exhausted to go. Nevertheless there had been in their minds a very pardonable jealousy of his paramount influence, and on his part an equally natural desire to stand aside till full authority was placed in his hands as President of that Republic which he had proclaimed and the people had welcomed. He believed that he alone could save Venice, and therefore he aspired to power little short of a Dictator's.[2]

At two in the afternoon of the 23rd, after a solemn service in S. Mark's, a great *arrengo* or folk-moot was held on the Piazza outside. First the obliging Patriarch blessed the Tricolor banner and those of the sister Republics of America and France. Then the Civic Guard and the sovereign people itself in full assembly, at the invitation of Mengaldo, elected by acclamation Daniele Manin to be President of the Republic, and afterwards his Cabinet Ministers to their several posts. As each name was read, a great shout affirmed the popular will. That night Manin moved into the Governor's Palace on the Piazza.[3]

During Manin's confinement to his bed on the night of the 22nd, a serious error had been committed by his rivals of the Municipal Council and by his friends Pincherle and Paolucci.

It was clear to all that the survival of Venetian liberty would largely depend on the command of the Adriatic, which in turn depended on the Austrian fleet then in the harbour of Pola. Its officers and men were most of them Italians in speech and sympathy, and the rest were Dalmatian Slavs, who disliked Austria and

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[1] *Medin*, p. 14.

[2] *P. de la F.*, i. pp. 140-141.

[3] *M.C.V.*, *Cavedalis MSS.*, i. p. 123. *Federigo*, p. xxix. *Gazz. Ven.*, March 24.

VI. THE LIMBS AS PROPULSIVE UNITS

The mechanical principles whereby the limbs of a tetrapod propel the body forwards relative to the feet are essentially the same as those by which they support it above the ground. In both cases a limb can operate as a strut or as a lever, exerting horizontal forces against the body and against the ground.

The limb as a propulsive strut

If the mechanical axis of a limb is retracted relative to the vertical, the limb can act as a propulsive strut provided that the horizontal component of its axial thrust is greater than the sum of all the posterior forces exerted on the body by the other limbs. On the other hand, a limb whose axis is protracted to the vertical applies, as a strut, a posterior force to the body and therefore acts as a brake. During a complete locomotory cycle the axis of a limb is first protracted and then retracted relative to the vertical and consequently its driving power as a strut is at first negative and later positive; the net propulsive effect depends on the amount of weight resting on the limb during the two phases of the limb's movement, and the locomotory mechanism is essentially the same as that of a punt when propelled by means of a pole.

If the limb girdle be regarded as part of the limb, the latter can act as a propulsive strut so long as the reaction of the ground against the foot passes through the centre of articulation of the body and limb, as in Fig. 42. The propulsive force applied to the body depends on the angle of inclination of the limb and on the axial thrust of the intrinsic limb muscles. As shown in §§ III and IV, a thrust of this type can be maintained so long as the extrinsic muscles remain inactive and the tensions of the intrinsic muscles are such that the moments about their respective joints are to each other in the same proportions as the distances of the joints from the axis of the limb.

It is obvious, however, that a limb cannot change its propulsive action as a strut without upsetting the balance of the vertical forces acting on the body (see p. 91).

The limb as a propulsive lever

As already explained in § III, the horizontal thrust applied to the body by a limb acting as an inclined strut can be increased or decreased by activity of the extrinsic muscles of the limb. Any tension developed by the retractor muscles enables the limb to apply an anteriorly directed force against the body and so enables the limb to act as a propulsive lever. As explained in § III, tension in a retractor muscle exposes the limb and body to equal but opposite couples, and if the foot is prevented from moving, the net effect is to propel the body forward and at the same time to tend to make it pitch about a horizontal transverse axis. The leverage of the limb is illustrated by Fig. 43, in which the axis (*HF*) of the limb is vertical, and the muscle (*M*) is attached to the pelvis (*P*) at *O*, the pelvis being attached to the vertebral column (*VC*) by an articulation (*S*) and a ligament (*L*). Tension in the muscle (*M*) exposes the limb (*HF*) to a couple (*Aa*, *Hh*, Fig. 48*b*), and if the foot (*F*) is prevented by moving, the reaction of the ground against the foot is $Fc\ (=\frac{Tm \sin \gamma}{l}$, see pp. 113–14). The resultant of the forces *Fc*, *Aa* and *Hh* is a force *Hb* (=*Fc*) acting anteriorly at the head of the limb as in Fig. 48*b* and tending to rotate the limb forwards about the foot. The pelvis (*P*) is exposed to the couple *Od*, *Hf* (see Fig. 48*c*) and the force *Hb*; the resultant of these forces is a force *Fc* acting at the foot, and consequently the pelvis will tend to rotate forwards about its centre of articulation *S* unless restrained by the ligament (*L*) acting posteriorly to *S*. The tension in this element must be such that its moment about *S* is equal but opposite to that of *Fc*. The resultant force exerted by the pelvis is therefore *Sj*, whilst the resultant forces acting on the vertebral column are *Sj*, *Dk* and *Sl* (Fig. 48*d*), the two latter forces representing the couple exerted by the sacral elements (*L* and *S*). It may be noted that when a limb is functioning as a propulsive lever in this way, the mechanism is essentially that of a canoe being propelled by means of a couple applied to a vertical paddle. The posterior pull exerted on the paddle by the lower arm of the operator represents the tension applied to the limb by a retractor muscle at its effective point of attachment (*A*) to the limb; the forward push against the head of the paddle which is exerted by the upper arm of the operator represents the forward thrust applied to the head of the limb by the pelvis; both the body of the tetrapod and the canoe are exposed to a turning couple.

Co-ordination of limbs as propulsive units

The total propulsive power of a limb is the sum of its action as a strut and as a lever, and the resultant effects of all four limbs represent the net force available for the propulsion of the body.

During every cycle of its movements each limb impresses on the body a relatively complex pattern of vertical and horizontal forces, the nature of which can only be determined by such experiments as those of Manter (1938). Manter's work shows that over the period of one complete cycle of comparatively slow limb movements, the hind limbs of a cat tend to propel the body against the resistance of the fore limbs. As a fore limb is brought into contact with the ground in the protracted position there is a brief period during which the retractor muscles are operative but to an extent insufficient to counteract fully the braking action of the limb as a strut. As the axis of the limb approaches the vertical position, however, the protractor muscles develop tension which increases as the limb is retracted behind the vertical.

Until retraction is nearly complete the braking power of the protractor muscles is greater than the driving power of the limb operating as a strut under the thrust of the intrinsic extensor muscles, and consequently the limb as a whole is acting as a brake. During the final phases of retraction, a converse condition exists for the driving power of the limb as a strut exceeds the braking power of the protractor muscles. The general effect of the extrinsic muscles is therefore to act as brakes on the limb operating as a strut; when the axis of the limb is protracted in front of the vertical the retractor muscles are active, and when the limb is retracted behind the vertical the protractor muscles are in action. Similar conditions exist in the case of the hind limb, but in this case the tension developed by the retractor muscles during the protracted phases of the limb movement may be more than sufficient to compensate the braking power of the limb operating as a strut. Similarly, during the retracted phases of a hind limb the tension of the protractor muscle is relatively small and consequently the limb can operate as a powerful propulsive strut. Over a complete cycle therefore, the fore limbs act as brakes whilst the hind limbs propel the body. This conclusion is illustrated, highly diagrammatically, by Fig. 44.

So far as vertical forces are concerned, Manter's data illustrate several points of general importance. First, even during slow locomotion the total vertical thrust of the limbs is at times substantially greater than the weight of the body and at other times it is less, indicating considerable vertical oscillations of the body relative to the ground. Secondly, the total vertical thrust of the two right limbs may be substantially different from that of the two left limbs, indicating either lateral displacements of the centre of gravity or rotation of the body about its longitudinal axis. On the other hand, the maximal value of the vertical thrust of each limb is approximately in phase with that of the diagonal limb.

Although it is quite clear that the body of a moving animal can never be entirely free from vertical or lateral oscillations, it is nevertheless of interest to consider the general conditions under which such oscillations are reduced to a minimum, thus yielding a type of locomotion which can be regarded as of maximum efficiency. From this point of view the co-ordination of limb effort should be such that the resultant propulsive force acting on the body should always be a horizontal force acting through the centre of gravity of the body, the latter being free from any tendency to rise, fall, pitch, roll or yaw. In order that these conditions should be completely fulfilled in respect to vertical disturbances two features are necessary: (i) not less than three feet must be on the ground at any one instant, and (ii) no foot can be lifted off the ground unless the centre of gravity of the body lies within the triangle of the other three feet.

If all the limbs step with equal frequency and length of stride there are six possible sequences in which they can move in relation to each other:

(1)	*RF*	*LF*	*LH*	*RH*
(2)	*RF*	*LF*	*RH*	*LH*
(3)	*RF*	*LH*	*LF*	*RH*
(4)	*RF*	*LH*	*RH*	*LF*
(5)	*RF*	*RH*	*LF*	*LH*
(6)	*RF*	*RH*	*LH*	*LF*

Taking the limiting case in which there are always three, but never four, feet on the ground simultaneously, each foot must be out of contact with the ground for a period of time equal to one-third of that during which it is in contact with the ground and thereby contributing towards the support and propulsion of the body. In other words, as one foot is being placed on the ground the foot which follows it in sequence will be lifted off the ground. For each of the six possible sequences, Fig. 51 shows the positions of the feet relative to the ground and to the centre of gravity of the body during one complete locomotory cycle. It will be noted that full stability is only provided by the diagonal pattern shown in sequence 3, and even then only if the centre of gravity of the body lies at a particular point relative to the shoulders or hips. Phases of mechanical instability inevitably occur in every other sequence. The suggestion thus arises that the diagonal pattern of limb movement, so characteristic of tetrapods, is essentially that which is necessary to maintain stability of the body in respect to vertical forces when the speed of locomotion is slow. This conclusion is confirmed by selecting, at random, a phase from one of the unstable sequences and considering the conditions under which the animal can progress. For example, in Fig. 51 the posture of the limbs is such as to place it in the rotary sequence *RL, LF, LH, RH*; the animal in this initial posture can be regarded as at rest supported by all four legs. Stability can be provided either by the triangle *BCD* or by *ABC*, and consequently either the right fore foot or the right hind foot can be lifted. If the right fore foot is lifted, and subsequent to this each foot in turn is lifted as and when it lies outside a triangle of support, either of the sequences I or II shown in Fig. 52 can occur. If, on the other hand, the animal starts the movement with its right hind foot sequence III (Fig. 52) must take place, it being assumed that a limb cannot be protracted beyond the points shown in the figures. In every case, it will be noted that the sequence of limb movements falls inevitably into the diagonal pattern:

Sequence I, Fig. 51 (I)	*RF*	*LH*	*LF*	*RH*	*RF*	*LH*	*LF*	*RH*
Sequence II, Fig. 51 (II)	*RF*	*RH*	*RF*	*LH*	*LF*	*RH*	*RF*	*LH*
Sequence III, Fig. 51 (III)	*RH*	*RF*	*LH*	*LF*	*RH*	*RF*	*LH*	*LF*

By drawing diagrams, similar to Fig. 52, for any other of the numerous limb positions displayed in Fig. 51, precisely similar conclusions are reached; in other words, for an animal whose centre of gravity is at G_2, stable locomotion at a slow speed is only possible if the sequence of limb movements conforms to the diagonal pattern. If diagrams are drawn for an animal whose centre of gravity is at G_1 or G_3 it will be found that stable motion is again only provided by the diagonal sequence, but in this case each limb must be retracted through a greater or lesser angle than is protracted, apart from this the same general conditions hold good. The whole situation can be summarized as follows: Stable locomotion can only occur when (i) a fore foot is placed on the ground at the moment at which the centre of gravity of the body lies on the diagonal joining the contralateral fore foot to the ipsilateral hind foot, (ii) a fore foot is lifted from the ground when the centre of gravity of the body lies posteriorly to the above diagonal, (iii) a hind limb is lifted from the ground when the centre of gravity of the body lies on the diagonal joining the ipsilateral fore foot to the contralateral hind foot, (iv) a hind limb is placed on the ground when the centre of gravity has passed over this diagonal. It must be clearly understood that this picture applies only to an animal moving slowly with three feet (neither more nor less) on the ground at any instant.

The main conclusion to be drawn from the above analysis is that, starting with any arbitrary posture of the limbs, slow but stable progression at uniform speed is only possible if the animal adopts a diagonal sequence of limb movements. To a surprising extent this conclusion can be demonstrated in the case of a spinal newt (Gray & Lissmann, unpublished data). If the nerve cord be severed immediately behind the medulla, a limited number of preparations exhibit clearly defined flexor reflexes in each of the four limbs; if a limb is mechanically retracted, it swings forward in a manner identical with the protractor phase of the limb of an intact walking specimen. If the spinal animal be passively towed along a straight line all the limbs exhibit stepping movements. When the animal is at rest, each limb can be placed in any partial degree of retraction, and if the preparation be then towed by means of a thread attached to its head, the diagonal sequence is rapidly established in all cases, irrespective of the original posture of the limbs (see Fig. 45).

It would be of interest to consider to what extent the distribution of body weight between the limbs forming the triangle of support provides reflex stimulation for the protraction of the fourth limb. At the same time, it is clear that other reflex mechanisms must be operative, particularly those associated with the passive retraction of limbs (Gray & Lissmann, 1940). Whatever be its underlying neuro-muscular mechanism, however, the diagonal pattern of limb movements is essentially an adaptation which permits slow and stable progression to occur.

The relationship of the above arguments to faster types of tetrapod movement will be considered elsewhere. In such cases varying degrees of instability occur in the sense that less than three feet may be on the ground simultaneously; nevertheless, in most cases the diagonal pattern of movement is preserved and is instrumental in reducing the duration of unstable phases to a minimum. Similarly, during very slow movement of some Amphibia and reptiles phases exist when all four feet are on the ground simultaneously. Under these conditions the equilibrium of the body in respect to vertical forces is determined by the same principles as those which exist when the animal is at rest (see § II), and any change in the vertical thrust of one limb must be accompanied by an appropriate readjustment of that of each of the other three (see also legend to Fig. 42).

The equilibrium of forces during movement

So long as the propulsive action of any one limb is equal but opposite to the static braking action of the remaining three limbs the animal remains at rest. If, on the other hand, the propulsive power of one or more limbs exceeds the static braking action of the remainder, the resultant force is free to accelerate the body against dynamic resistances either internal or external to the body. For all moderate speeds, external forces, such as wind resistance, can probably be regarded as small in comparison with the internal resistances which are due to the forces required to stretch the extrinsic muscles of limbs which are acting as brakes. If it is possible to imagine the body of an animal moving forward slowly at constant speed through still air, the propulsive forces and couples applied to the body by the active limbs would be equal but opposite to the restraining forces and couples applied by other limbs acting as brakes. If, on the other hand, the propulsive forces were greater than the restraining forces, the body would be accelerated and the energy of the propulsive limbs used to increase the kinetic energy of the body and not to stretch other extrinsic muscles.

From a functional standpoint it is important to remember that an animal cannot accelerate its body without disturbing the balance of vertical forces acting on it. The significance of this fact is illustrated by Fig. 46, in which the animal is assumed to be standing at rest (Fig. 46*a*) with all its limbs vertical and with the limb loading symmetrical on the two sides of the body. If the retractor muscles of the two hind limbs now develop tension sufficient to exert a total anterior force F against the pelvis the body is thereby exposed to a turning couple C_1, C_2 (Fig. 46*b*) whose moment is equal to that of the combined tensions of the right and left hind-limb retractor muscles about the hip joints, and also equal to Fh, where h is the height of the limb. This couple induces an upward force $D_2 \left(= \frac{Fh}{a+b}\right)$ at the shoulders and a similar downward force D_1 at the hips; there is therefore a transfer of weight from the front to hind limbs, the amount of which varies directly with the height of the body and inversely as the distance between the hips and shoulders. As the animal starts from rest, therefore, additional strain falls on the intrinsic extensor muscles of the hind limbs and less on those of the fore limbs. In an extreme case (when $F = Wb/h$) the whole of the weight rests on the hind limbs and none on the front limbs. This fact is of functional interest for two reasons: (i) The reduction of the weight resting on the front limbs reduces their power of acting as propulsive units since their horizontal thrust cannot exceed a value which depends directly on the weight being carried. A cursorial animal attempting to accelerate rapidly with its front limbs (Fig. 46*d*) runs the danger of these limbs slipping on the ground, whereas an animal accelerating with its hind limbs does not face this danger. (ii) If the turning couple exerted on the body by the retractor muscles of the limb exceeds that which can be compensated by a transfer of weight from front to hind limbs, the axis of the body will rotate upwards about the hips as it moves forwards; the front limbs are thereby lifted off the ground and the animal springs forwards. In other words, by adjusting the posture of the body and the pull of the retractor muscles until the line of action of the reactions from the hind feet passes through or in front of the centre of gravity of the body an animal can jump forwards. The nearer are the hind feet to the centre of gravity the more readily is this condition reached. In forms adapted for jumping (e.g. frogs) the centre of gravity lies well back towards the hip, and the typical 'jumping off' position of a cursorial mammal is characterized by a preliminary backward movement of the centre of gravity of the body towards the hind feet. Conversely, an animal preparing to spring forward without raising its front feet off the ground, starts by moving its centre of gravity well forward from the hips in order to generate a maximum forward drive without wasting energy in lifting the front end of the body off the ground. (iii) In animals whose centres of gravity normally lie well back towards the hips a relatively slight effort on the part of the retractor muscles is sufficient to lift the fore-quarters off the ground, and, if the pull of the retractors is adequate, the body pitches upwards about the hips until its centre of gravity lies over the hind feet; such an animal can remain bipedal as long as the body is held in this position, e.g. a horse when rearing, or *Chlamydosaurus* and similar lizards when moving rapidly.

As already mentioned, an animal which is moving slowly and at uniform speed through still air over a horizontal surface uses the energy of its muscles almost exclusively to overcome resistances internal to the body; the only effective external force acting on the body is gravity, and against this force the animal does no work. On the other hand, when an animal is moving against an external horizontal restraint due to high wind resistance or to an attached load, it is subjected to a backward turning couple which tends to throw the weight of the animal on to the hind limbs, and the conditions are the converse of those in Fig. 11; an equitable distribution of weight is effected by keeping the centre of the body well forwards towards the front feet, thereby enabling the front limbs to function as effective propulsive agents.

Role of the vertebral muscles during locomotion

As explained in § V any vertical force exerted by the extrinsic limb muscles against the body affects the total bending moment about each of the vertebral joints. Thus, if the hind limbs are acting as propulsive levers, the pull of the retractor muscles acting behind the acetabulum tends to arch (flex) the back, particularly in its sacral and lumbar regions, whilst a similar flexing action is exerted on the thoracic region by the protractor muscles of the fore limb if these muscles are operating as brakes. On the other hand, if the retractor muscles of the front limbs are driving against the braking action of the protractors of the hind limbs, the pull of all the muscles is applied between the shoulders and hips and consequently the back tends to sag (extend). In most tetrapods the musculature of the hind limbs is more powerful than that of the fore limbs and consequently the dorsal muscles, particularly in the lumbar region, must respond by powerful contraction when the animal is moving rapidly forwards. Precisely the same principles operate when an animal is pulling a heavy load or walking uphill and therefore 'putting its back into its work'.

In the case of a typical cursorial mammal the propulsive drive is normally controlled by the muscles of the limb, whilst the axial musculature of the back co-operates by providing the body with adequate rigidity. In lower tetrapods, however, the propulsive drive may originate in the vertebral musculature whilst the retractor muscles of the limbs co-operate by giving adequate rigidity to the shoulder and hip joints. This condition (see Fig. 47) is sometimes regarded as the primitive tetrapod condition. In Fig. 47*a* (i) the right fore and left hind limbs are protracted and are in contact with the ground whilst the axis of the body is curved with marked convexity to the right side. If the axial musculature of the right side of the body contracts isotonically (Fig. 47*a* (i) and (ii)), posteriorly directed forces will be exerted by both right fore and left hind feet against the ground provided the retractor muscles of both these limbs hold them isometrically rigid at the shoulder and hip. Alternatively (Fig. 47*b*), a posterior force can be exerted against the ground by isotonic contraction of the retractor muscles of the limbs provided the axial musculature gives sufficient isometric rigidity to the body. In practice the two patterns of movement appear to be superimposed on each other when a newt is moving at relatively high speed (Fig. 47*c*). In all cases the axial musculature of the back forms an integral part of the locomotory mechanism.

SUMMARY

1. As a propulsive mechanism, the limb of a tetrapod functions as an extensible strut and as a lever. In so far as it acts as a propulsive strut, the limb is extended by its own intrinsic musculature and the mechanism of locomotion is essentially that of a punt when propelled by a pole. In so far as the limb functions as a lever, it is operated by its extrinsic musculature and the mechanism is essentially similar to that of a canoe propelled by a two-handed paddle, the distal end of which remains fixed.

2. In so far as it operates as a strut any limb in a retracted posture exerts a propulsive action; a similar limb in a protracted posture exerts a braking action. In both cases the horizontal force acting on the body depends on the axial thrust of the limb's musculature and on the angle of inclination of the limb. The propulsive or braking action of a limb operating as a strut can be increased or decreased by the operation of the limb as a lever. During each cycle of movement a limb alternately acts as a brake or as a propulsive element; over the whole cycle the fore limbs of a cursorial mammal probably exert a resultant braking action whereas the hind limbs have a resultant propulsive action.

3. The diagonal co-ordination of limb movements seen in nearly all tetrapods enables the limbs to propel the body forwards with a minimum of uncompensated pitching or rolling couples. It is in fact the only pattern of movement which during slow movement enables the body to be in static equilibrium with its own weight at all phases of the movement; the animal can therefore come to rest at any point without falling over.

4. The forces acting on the body and at the feet of a moving animal are discussed, and brief reference made to the relative importance of front and hind limbs as organs of propulsion.

5. The locomotory significance of the axial musculature of the vertebral column is briefly considered.

APPENDIX I

Equilibrium of limbs in respect to their own weight

Without introducing any new mechanical principles it is possible to repeat the analysis given in this section, taking into account the weight of the limb itself. Apart from the forces exerted on it by extrinsic muscles there are three forces acting on each limb: (i) the force exerted by the body against the proximal end of the limb, (ii) its own weight acting at its centre of gravity, (iii) the reaction of the ground against the foot. In the simplest mechanical case (Fig. 48*a*) the lines of action of the first and second of these forces are identical and the sum of the two forces is equal and opposite to the reaction of the ground against the foot. In life, such conditions may seldom, if ever, be satisfied, but an approximation is reached in graviportal types similar to the elephant. In the majority of cases, the lines of action of the body and limb weights are not coincident; in Fig. 48*b* the lines of action in the hind limb, nevertheless, fall within the surface of contact of the foot with the ground, and each can be compensated by an equal and opposite reaction from the ground. The resultant of the two individual ground reactions is a single force (V_4, Fig. 48) (equal to the sum of the body and limb weights) acting vertically upwards at a common centre of pressure (D) of the foot. The common centre of pressure of the foot lies between the lines of individual action of the body and limb weights at a point (D) about which the moments of the two latter forces are equal and opposite to each other. In other words, the couple exerted by the weight of the limb is equal to that of the weight of the body but is acting in the opposite direction. Any alteration in the loading of the limb will upset this equilibrium. If the weight of the body being supported by the limb be increased, the centre of pressure of the foot will move towards the heel, if the load is decreased it will move towards the toes. If the horizontal displacements of the centre of gravity (g_4) of the right hind limb (Fig. 48*c*) from a vertical axis through the head of the femur be x and y, the corresponding co-ordinates (x_1 and y_1) of the centre of pressure of the foot are

$$\frac{xL_4}{L_4+W_4} \text{ and } \frac{yL_4}{L_4+W_4},$$

where L_4 is the weight of the limb and W_4 the support which the limb is providing for the body.

If the resultant of the body and limb weights falls outside the surface of contact of the foot with the ground the resultant couple exerted on the limb by virtue of its own weight and that of the body must be compensated by methods essentially the same as those which compensate the limb against the weight of the body.

Taking a generalized case, a limb can only be in longitudinal equilibrium when the sum of the turning moments about the foot is zero,

$$M_1+M_2+M_3+M_4=0,$$

where M_1 = moment of the weight of the body, M_2 = moment of the weight of the limb, M_3 = moment of the thrust of the vertebral column, M_4 = moment of the extrinsic muscles of the limb. Similar conditions apply to equilibrium in respect to moments acting in a transverse vertical plane.

APPENDIX II

Operation of a limb as a lever

The action of an extrinsic muscle acting about a hip joint can be illustrated quantitatively from Fig. 21. The tension (T_B) exerted by the muscle against the body (Fig. 21*a*) can be resolved into $OP = T_B \sin\beta$ and into $OQ = T_B \cos\beta$, whilst the latter can be replaced by $RH = T_B \sin\beta$ and by $HS = T_B$. The couple (OP, RH) operating on the body has a moment $T_B \sin\beta OH$, whilst that (HS, T_L) operating on the limb has a moment $T_L z = T_L m \sin\gamma$. Since $OH = \frac{m \sin\gamma}{\sin\beta}$, the moments of the couples acting on the body and on the limb are equal but opposite to each other; and both are equal to the moment Tz of the muscle's tension about the centre of rotation of the joints.

The ability of the limb to exert horizontal forces against the body and against the ground are illustrated in Fig. 21*b*. The couple (HS and T_L) exerted by the muscle on the limb can be resolved into compression forces ($T_L \cos \gamma$) acting along the upper part of the limb's axis and into components (HV and AW; $=T_L \sin \gamma$) acting normally to this axis. The force AW exerts a reaction $HL\left(=T_L \frac{l-m}{l} \sin \gamma\right)$ at the hip and a reaction $DM\left(=T_L \frac{m \sin \gamma}{l}\right)$ against the ground. The resultant force ($HV-HL$) acting at the hip is $HX\left(=T_L \frac{m \sin \gamma}{l}\right)$. The horizontal component of HX represents a posterior pull exerted on the body, whilst the vertical component of HX contributes towards the support of the body. If the limb is to be in equilibrium when the muscle is in action, a force equal but opposite to HX must be applied by the body to the head of the femur; alternatively, the muscle enables a limb to compensate a posterior thrust from the body by operating the limb as a lever whose fulcrum is the foot.

It is useful to note that if the leverage of the protractor muscle shown in Fig. 21 is accompanied by a relatively small but definite axial tension from the intrinsic extensor muscles (DP and HY in Fig. 49), the resultant forces exerted against the body (HG) and against the ground (DR) are horizontal. In other words, provided that there is slight but adequate co-ordination between the axial and extrinsic muscles, it is possible to regard the vertical forces exerted by the limb as being under the control of the axial musculature whereas variation in the horizontal forces exerted by the limb is under the control of the extrinsic muscles. It must be remembered, however, that the division of the musculature into intrinsic axial and extrinsic rotary units is a purely functional conception; for its relationship to the morphology of the muscles see p. 103.

The ability of an extrinsic muscle to compensate both body and limb against couples imposed on them by the weight of the body is further illustrated in Fig. 50. If the resultant of the weight of the whole body and the vertical thrusts of the three remaining limbs represents a force VB exerted on the body, the external forces acting on the whole body will be in equilibrium provided that a vertical reaction ($V_4=V_b$) is acting at the foot. In its capacity as a strut the limb applies a vertical thrust (VH) at the hip together with an anterior horizontal thrust ($H_4=V_4 \tan \alpha$) against the pelvis and consequently the body is exposed to a forward thrust ($H_4=V_4 \tan \alpha$) and a couple $V_4 x$. If a protractor muscle develops a tension (T), the moment of the couple (HR, OP, Fig. 50*b*) it exerts on the body is Tz (where z is the working distance of the muscle from the joint). Equilibrium between the muscle and the weight of the body will exist when $V_4 x=Tz$. The posterior horizontal force exerted (owing to the muscle) by the body against the head of the limb is Tz/h, and if $T=V_4 x/z$, the posterior thrust of the body against the hip is $V_4\,(x/h)=V_4 \tan \alpha$. In other words, the posterior thrust exerted by the muscle on the body is equal to the anterior horizontal thrust exerted by the limb in its capacity as a strut supporting the weight of the body.

Just as the couple provided by an extrinsic muscle can compensate a vertical couple applied to the limb by the weight of the body, so also it can compensate a horizontal couple due to a thrust, or pull, exerted on the limb by the vertebral column.

APPENDIX III

Coordination of limbs acting as struts and levers

The general picture of limb co-ordination can be illustrated quantitatively as follows. In order that the animal in Fig. 22 (whose limbs are symmetrically loaded on the two sides of the body) should not pitch about the front feet

$$V_1=\frac{W}{2}\,\frac{x_4+b}{a+b+x_1+x_4}, \quad V_4=\frac{W}{2}\,\frac{x_1+a}{a+b+x_1+x_4}.$$

The two right limbs both acting as struts each exert horizontal thrusts (H_1, H_4) against the ground

$$H_1=\frac{W}{2}\left(\frac{x_4+b}{a+b+x_1+x_4}\right)\frac{x_1}{h} \quad \left[\frac{x_1}{h}=\tan \alpha\right],$$

$$H_4=\frac{W}{2}\left(\frac{x_1+a}{a+b+x_1+x_4}\right)\frac{x_4}{h} \quad \left[\frac{x_4}{h}=\tan \beta\right].$$

The difference between H_1 and H_4 $\left(=\frac{W}{2}\,\frac{(ax_4-bx_1)\,h}{a+b+x_1+x_4}\right)$ must be made good by protractor muscular activity as in Figs. 22 *a* and *b*, and the total moments exerted by these muscles must be $\frac{W}{2}\left(\frac{ax_4-bx_1}{a+b+x_1+x_4}\right)$.

If the vertical thrust (V_4) of the right hind limb is now reduced to $\frac{W}{2}\,\frac{x_1}{x_1+x_4}$ (Fig. 23*a*), that of the right fore (V_1) limb becomes $\frac{W}{2}\,\frac{x_4}{x_1+x_4}$. Both these limbs can now support the right side of the body as struts since

$$H_1=\frac{W}{2}\,\frac{x_4}{x_1+x_4} \tan \alpha,$$

$$H_4=\frac{W}{2}\,\frac{x_1}{x_1+x_4} \tan \beta,$$

$$H_1=\frac{W}{2}\,\frac{x_4}{x_1+x_4}\,\frac{x_1}{h}=H_4.$$

On the other hand, the body is exposed on its right side to a pitching moment (M) about the front feet:

$$M=\frac{W}{2}\,(a+x_1)-V_4\,(a+b+x_1+x_4)$$

$$=\frac{W}{2}\left[\frac{ax_4-bx_1}{x_1+x_4}\right].$$

In other words, the centre of pressure of the two right feet lies at a point (G_1) on the ground anteriorly to G

Patriots Proclaiming the Republic of S. Mark, March 22, 1848

(On the further side of the canal, a man in a top hat is carrying a winged lion, the emblem of S. Mark and of the Republic)

his colleagues to act with them for this purpose, on the ground that he was too ill and tired to leave his bed that night.[1] No doubt they had invited him to join them, and no doubt he had been too much exhausted to go. Nevertheless there had been in their minds a very pardonable jealousy of his paramount influence, and on his part an equally natural desire to stand aside till full authority was placed in his hands as President of that Republic which he had proclaimed and the people had welcomed. He believed that he alone could save Venice, and therefore he aspired to power little short of a Dictator's.[2]

At two in the afternoon of the 23rd, after a solemn service in S. Mark's, a great *arrengo* or folk-moot was held on the Piazza outside. First the obliging Patriarch blessed the Tricolor banner and those of the sister Republics of America and France. Then the Civic Guard and the sovereign people itself in full assembly, at the invitation of Mengaldo, elected by acclamation Daniele Manin to be President of the Republic, and afterwards his Cabinet Ministers to their several posts. As each name was read, a great shout affirmed the popular will. That night Manin moved into the Governor's Palace on the Piazza.[3]

During Manin's confinement to his bed on the night of the 22nd, a serious error had been committed by his rivals of the Municipal Council and by his friends Pincherle and Paolucci.

It was clear to all that the survival of Venetian liberty would largely depend on the command of the Adriatic, which in turn depended on the Austrian fleet then in the harbour of Pola. Its officers and men were most of them Italians in speech and sympathy, and the rest were Dalmatian Slavs, who disliked Austria and

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[1] *Medin*, p. 14.

[2] *P. de la F.*, i. pp. 140-141.

[3] *M.C.V.*, *Cavedalis MSS.*, i. p. 123. *Federigo*, p. xxix. *Gazz. Ven.*, March 24.

reconquest of Venetia. Gyulai sent immediate warning to the authorities at Pola. On the 26th of March the guns of the forts were trained on the warships in the harbour; the Italian sailors were sent, free and rejoicing, to Venice, and the officers brought on board their ships to Trieste, where most of them resigned their commissions. The business of building up fresh crews for the vessels thus saved for the Imperial service, was taken in hand along the Dalmatian coast, and in the course of the next year was gradually effected.

It has generally been assumed by Italian writers that if the order for the fleet to come to Venice had gone straight to Pola on the 23rd, it would have been obeyed. This is possible, but it is not certain. The Naval Commandant at Pola, Buratovich, was loyal to his flag, and when, forty-eight hours later, Captain Fincati came from Manin with the delayed message, he had him arrested and flung into the dungeons of Lubiana. The question is, would Buratovich have been able to treat a messenger from Venice in that fashion if he had arrived at Pola on the 23rd before the warning from Trieste, or would the crews at that earlier date have mutinied successfully and carried the fleet to Venice. No certain answer can be given.[1]

Meanwhile the whole lagoon, with its numerous forts, their thousand cannon and their military stores, had safely passed into the hands of the Republic. The largest and most important fortress was Marghera, or Malghera, built by Napoleon amid the marshes of the *laguna morta* as the bridge-head of Venice. The new railway to Mestre passed close by its walls. The free communications of the Republic with the mainland depended on securing it. It was indeed to have been given

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[1] *Benko*, pp. 125-126, 130-133. *Marchesi*, pp. 135-136. *Marchesi*, 70 *anni*, pp. 115-116, 224. *Cusani*, pp. 29-30. *Blue Book*, ii. pp. 286, 419. *P. de la F.*, i. pp. 111-113. *Medin*, pp. 15-19. *Gonni*, p. 222. *E. and F.*, p. cxl, note. *Errera*, pp. 36-38. Address to Tommaseo, Aug. 2, 1849, in *Misc. B.M.*, 1852, *e.* 10. *Rovani*, p. 39. *Raccolta*, i. pp. 562-564, 604.

Padua by the Austrians under d'Aspre, made a fresh convention with Manin, under which the men of the Kinsky were shipped to Trieste but with their muskets in their hands.[1]

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[1] *Ulloa*, i. pp. 63-64. *Blue Book*, ii. p. 270. *M.C.V.*, *Cavedalis MSS.*, i. pp. 124-125. *Marchesi*, p. 134. *Radaelli*, *Manin*, p. 63. In the Museo Fantoni, Vicenza, is a contemporary sketch of the gunboats, each with its single cannon in the bows trained on the Zattere barracks.

Radetzky by the citizens of Milan gave them freedom and glory, but left them divided by embittered factions and with no leader of commanding eminence; but Venice, on the departure of the Austrians, though not wholly of one mind, was united in admiration and love for Manin.

The liberation of Milan at once involved the liberation of the whole Lombard Province, and the revolution in Venice was the direct cause of a similar revolution in the Venetian *terra firma*. By the end of the month the Austrian power in Italy was confined behind the Isonzo on one side, and within the district of the quadrilateral fortresses on the other.[1]

Very different would the situation have been if, while Milan and the Lombards expelled Radetzky, Venice and the Venetian cities had allowed themselves to be amused by Austria's promise of constitutional rights. Tommaseo might descant to admiring audiences on the true nature of constitutional liberty and on the amity of peoples, but if meanwhile the regiments, fortresses, arms and munitions in all Venetia had remained at the disposal of Radetzky, if Venice had continued to be the place of landing from Trieste for reinforcements and stores from the interior of the Empire, Lombardy would have been reconquered and Piedmont crushed before the end of April.[2] The history of 1848 in Italy would then have seemed very different in the retrospect. Instead of an irrepressible outburst of the spirit of the whole nation, ending after a prolonged struggle in a tragedy fraught with noble memories and high promise, there would

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[1] The quadrilateral fortresses were Verona, Peschiera, Mantua and Legnago, guarding the 'square' of the territory between the Adige and the Mincio, the debouchment into Italy of the great Brenner Pass from Innsbruck and Vienna. See Map I. below.

[2] For what Lt. Marshal Hess himself thought on the importance of the Venetian revolution, see *Feldzug*, i. p. 53. His rival, Lt. Marshal Schoenhals, thought just the same: 'If Venice had held out like Mantua and the other strong places, Radetzky would have fallen on Charles Albert before he had had time to concentrate on the Mincio, and would have destroyed him. That was our plan, which the disaster at Venice prevented.' *Schoenhals*, p. 115. See also *Ellesmere*, p. 40.

(lying vertically under the centre of gravity of the body) by a distance $p=\frac{ax_4-bx_1}{x_1+x_4}$. To neutralize this pitching moment about the front limbs, the centre of pressure of the left side must lie at G_2 (Fig. 23*b*) and the distribution of weight between the two left feet (V_2 and V_3) must be

$$V_2=\frac{W}{2}\left(\frac{x_4+b-p}{a+b+x_1+x_4}\right), \quad V_3=\frac{W}{2}\left(\frac{x_1+a+p}{a+b+x_1+x_4}\right).$$

If both these limbs act as struts the horizontal thrusts imparted to the body are

$$H_2=\frac{W}{2}\left(\frac{x_4+b-p}{a+b+x_1+x_4}\right)\frac{x_1}{h},$$

$$H_3=\frac{W}{2}\left(\frac{x_1+a+p}{a-b-x_1-x_4}\right)\frac{x_4}{h},$$

$$H_3-H_2=\frac{Wh}{2}\left[\frac{ax_4-bx_1+p\,(x_1-x_4)}{a+b+x_1+x_4}\right].$$

To provide a posterior horizontal force H_3-H_2 the moment (M_1) of the protractor muscles of the left hind limb must be

$$M_1=\frac{W}{2}\left[\frac{ax_4-bx_1+p\,(x_1+x_4)}{a+b+x_1+x_4}\right].$$

Substituting for p

$$M_1=W\left[\frac{ax_4-bx_1}{a+b+x_1+x_4}\right].$$

Clearly the moment of the left hind protractor muscles is the same as the combined moments of the right and left side protractors shown in Figs. 22 *a*, *b*. In other words, by altering the vertical loading of the limbs the strain on the extrinsic muscles of the right side has been shifted completely on to those of the left side.

GENERAL SUMMARY

1. From a mechanical point of view the organization of a tetrapod's body is essentially that of a segmented, flexible and overhung beam supported by four limbs; each limb can operate both as a strut and as a lever. A substantial number of physiological and morphological facts receive rational explanations when subjected to mechanical considerations imposed by the animal's own weight.

2. The general conditions under which a tetrapod can use any particular limb for purposes other than the support or propulsion of the body depends on the positions of the four feet relative to the centre of gravity of the body.

When a cursorial tetrapod is standing on four legs, the contribution which any one limb makes towards the support of the body can vary within limits which can be defined in terms of (i) the weight of the body, (ii) the positions of the centres of pressure of the four feet relative to the centre of gravity of the body. If the thrust of one limb is known that of each of the other three can be calculated.

3. If the vertical thrust of any one limb be increased, there must be a simultaneous increase in the thrust of the diagonally situated limb and a decrease in the thrusts of both feet situated on the other diagonal. The reflex myotactic response of mammalian extensor muscles to mechanical stretch provides an adequate mechanism for ensuring effective co-ordination between the vertical thrusts exerted by each of the four limbs. The physiological response of all the limbs to flexor stimulation of any one of them conforms to the mechanical requirements for stability; it probably represents an extreme instance of readjustment of limb thrusts.

4. In bipedal forms a quadrilateral of support is provided by the heel and toes of the two feet: the conditions of stability are essentially the same as in tetrapods.

5. If a tetrapod stands on an inclined slope (in a posture similar to that normally adopted on a horizontal surface) the extent of the resultant redistribution of weight between front and hind feet is inversely proportional to the relative length of body and limbs. A long low body is a mechanical adaptation to a scansorial habit. The typical postures adopted by tetrapods when standing on steep slopes (viz. either with the axes of the limbs retracted or the back horizontal and the hind limbs flexed) conform to the principle that the distribution of the total weight of the body between the various limbs should approximate closely to that characteristic of the animal standing in a normal posture on a horizontal surface. The same principle applies to the posture adopted by an animal when exposed to restraint by an extraneous horizontal force.

6. The limbs of a tetrapod can contribute towards the support of the body either in the capacity of struts or in the combined capacity of struts and levers. When acting as a strut a limb exerts forces along its own mechanical axis only, the moment of the muscular tensions operating about the hip or shoulder joint being zero. When a limb is acting as a lever the limb exerts both against the body and against the ground forces at right angles to its mechanical axis; it is able to do so by means of the muscles whereby the limb is attached to the body.

7. When a limb is acting as an inclined strut the couple which is exerted on it by the weight of the body must be compensated by a couple due to (i) a horizontal force exerted on its proximal end by the body, and (ii) friction or other horizontal force acting at the foot. The horizontal force acting on the proximal end of the limb represents the resultant horizontal force exerted on the body by the three other limbs. Instances are described of two or more limbs co-operating, either as longitudinal or transverse struts, for the support of the body.

8. When subjected to tension from a muscle originating on the body, a limb operates as a lever and exerts equal but opposite horizontal forces against the ground and against the body. At the same time it exposes the body to a turning couple whose moment is equal to that of the muscle's tension about the centre of rotation of the joint.

By means of the muscles which operate between the body and the limb an animal can control the horizontal forces exerted by a limb against the body and against the ground.

9. The muscular co-ordination between two or more limbs, acting as levers, is described. For any given position of the feet relative to the centre of gravity of the body, the minimum total muscular effort required to maintain equilibrium is always the same. The distribution of this effort between the muscles of the various limbs can, however, be varied between relatively wide limits, either by changing the tension of one or more extrinsic muscles or by changing the distribution of weight between the feet by a change in the axial thrust of the limb. The whole of the limb musculature of the animal must be regarded collectively as one functional unit.

10. The particular pattern of muscular activity adopted for any given posture and loading of the limbs is probably such that the strain is distributed between the relevant muscles in proportion to their ability to develop and sustain tension.

11. The quantitative muscular effort required for the stabilization of any limb joint depends on the magnitude and direction of the reaction of the ground against the foot and on the distance of the centre of rotation of the joint from the line of action of this force. Since the reaction of the ground against the foot is under the control of the animal, the latter can continue to stand whilst varying the strain falling on any one intrinsic or extrinsic muscle provided it readjusts the activity of all the others not only in the same but also in all the other limbs. Instances are given of the co-ordination of the musculature in the four limbs of an animal standing at rest.

12. The digitigrade habit is associated with a posture of limbs in which the line of action of the body weight passes anteriorly to the distal metacarpal or metatarsal joints.

13. The forces exerted on joints by curvilinear muscles are discussed. In general the centripetal pressure exerted by the muscle is equal and opposite to the resultant longitudinal thrusts exerted by the two main bones composing the joint. Brief reference is made to multi-joint muscles.

14. The nature of the strains imposed on the vertebral column by the weight of the body and by the forces exerted by the ground against the feet of a tetrapod are discussed.

15. The mechanical picture presented by a tetrapod is essentially that of a flexible overhung beam supported by four elastic legs; only under very special circumstances can the body be compared to two balanced centilevers.

16. By means of the muscles of its limbs an animal can increase or decrease the strain falling on its vertebral musculature or vice versa.

17. As a propulsive mechanism, the limb of a tetrapod functions as an extensible strut and as a lever. In so far as it acts as a propulsive strut, the limb is extended by its own intrinsic musculature and the mechanism of locomotion is essentially that of a punt when propelled by a pole. In so far as the limb functions as a lever, it is operated by its extrinsic musculature and the mechanism is essentially similar to that of a canoe propelled by a two-handed paddle, the distal end of which remains fixed.

18. In so far as it operates as a strut any limb in a retracted posture exerts a propulsive action; a similar limb in a protracted posture exerts a braking action. In both cases the horizontal force acting on the body depends on the axial thrust of the limb's musculature and on the angle of inclination of the limb. The propulsive or braking action of a limb operating as a strut can be increased or decreased by the operation of the limb as a lever. During each cycle of movement a limb alternately acts as a brake or as a propulsive element; over the whole cycle the fore limbs of a cursorial mammal probably exert a resultant braking action whereas the hind limbs have a resultant propulsive action.

19. The diagonal co-ordination of limb movements seen in nearly all tetrapods enables the limbs to propel the body forwards with a minimum of uncompensated pitching or rolling couples. It is in fact the only pattern of movement which enables the body to be in static equilibrium with its own weight at all phases of the movement; the animal can therefore come to rest at any point without falling over.

20. The forces acting on the body and at the feet of a moving animal are discussed, and brief reference made to the relative importance of front and hind limbs as organs of propulsion.

21. The locomotory significance of the axial musculature of the vertebral column is described.

Summaries of §§ I–VI will be found on pp. 97, 102, 106, 108, 113.

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EXPERIMENTAL PRODUCTION OF ATROPHIED AND PARTLY DEFICIENT LIMBS (PHOCOMELIAS) IN THE AXOLOTL EMBRYO*

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(With Plates 4 and 5)

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The many different types and variations of deformities of limbs in animals and man are usually described and grouped from the morphological or genetical point of view. A very few biological experiments have been done for the purpose of elucidating the causes which produce special types of deformities in the embryo.

In an earlier paper (Brandt, 1940) the author has shown that supernumerary limbs such as reduplications and some higher degrees of multiplications can be produced experimentally in the frog embryo by special methods. Contrasting with the group of supernumerary limbs is the group showing stunted growth or even absence of the limb as a whole. On the other hand, there may be absence of parts of a single limb such as phocomelias and stunted growth or abnormal position of the digits.

The following experiments are the first which have been published concerning the problem of experimental production of various types of atrophied limbs in the amphibian embryo.

MATERIAL AND METHODS

The methods of production of atrophied or partly deficient limbs in the embryo are different from those which can be applied to the adult animal. The removal of parts of a primordium of the limb or of the whole limb bud in the amphibian embryo is followed by its perfect regeneration. The larva which develops later from such an embryo shows no trace of the operation as a rule, but it was found (Brandt, 1924) that after removal of the limb bud in the embryo newt there was no regeneration of the limb in 6% of the cases.

Implantation of foreign bodies, such as small pieces of coal, wax, or glass, into the primordium of the limb bud is followed either by their elimination in the course of a few hours after the operation or by a large hydrops of the affected area and its adjacent parts which usually causes death of the animal.

The author succeeded in implanting small pieces of ectoderm cut from the tip of the tail of another embryo. This implant was never eliminated and readily healed wherever it was implanted. It was possible to apply the implant at any given spot on the limb bud, and produce different types of atrophied limbs.

Three different methods of implantation were used:

(1) Deep implantation into the base of the primordium of the limb, allowing the ectoderm of the host to grow over the implant.

(2) Superficial implantation into the outer zone of the primordium after removal of a part of the overlying ectoderm of the host.

(3) Removal of a vertical zone of ectoderm from the limb bud running through the whole length of the limb bud and replacing it by the ectoderm of the donor embryo.

Date of operation	No. of animals	Average of the length of the embryo mm.	Average of the breadth of the embryo mm.	Average of the length-breadth index of the embryo
16 May	5	4·1	1·5	3
17 May	13	5·7	1·5	2·6
18 May	6	6·5	1·5	2·3
19 May	8	6·6	1·5	2·2
20 May	2	7·9	1·65	2·1
22 May	9	8·5	1·8	2·1
23 May	4	9·6	1·95	2·0
24 May	4	9·8	2·0	2·0
25 May	4	10·0	2·0	2·0
26 May	2	10·0	2·0	2·0
27 May	3	11·5	2·0	1·7

In methods (2) and (3) a part only of the ectoderm of the limb bud is replaced by the ectoderm of the donor. If the whole extent of the ectoderm of the limb bud is replaced by the ectoderm of the tail, then a tail-like structure develops and regeneration of the limb is suppressed.

The three methods were employed on sixty embryos of the axolotl (*Amblystoma mexicanum*) in

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the straight tail-bud stage and they refer to the cases 950–1010 of the author. For an exact determination of the stage of development, the length of each embryo from the vertex to the tip of the tail and the greatest breadth of the body of the embryo were measured by the aid of an oculo-micrometer; the average of the length-breadth index of the operated embryos was calculated at each day.

The above table shows the details of measurements and will be referred to in the description of the experiments.

DESCRIPTION OF THE EXPERIMENTS

Embryos operated after method 1 (*deep implantation*)

Out of twenty-nine operated embryos seven survived:

Exp. 959, operation 16 May. Six weeks after the operation the right limb consisted of a short pointed stump; the left limb showed rudimentary digits. After a further 4 weeks the right stump did not show any finger-like processes, and it remained at this stage of development without showing any further growth during the next few weeks. The left limb was normal and well differentiated.

Exp. 960, operation 17 May. The right limb bud developed a short stump with a swelling at its caudal border 3 weeks after operation. No further differentiation took place later.

Exp. 963, operation 17 May. Twenty-six days after the operation a normal right limb was fully developed, the length of its upper arm was 1·3 mm., the length of its forearm was 2·4 mm. The total length of the operated left limb was 0·6 mm., and its distal end possessed instead of fingers a series of four very small finger-like elevations (Pl. 4, fig. 1).*

Exp. 975, operation 17 May. Six weeks after the operation the normal left limb was fully developed; the operated right limb consisted of a pointed stump, the proximal end of which was swollen and oedematous (Pl. 4, fig. 2).

Exp. 989, operation 22 May. The limb 3 weeks after the operation was represented by a stump, the distal end of which was bent ventrally. In the course of the next 5 weeks no further growth took place, and the distal end of the limb persisted as a knob-like protuberance.

Exp. 990, operation 22 May. Four weeks after the operation the operated limb was shorter than normal and showed an abnormal position of its digits. The distal end of the limb was bifurcated and showed two digits which were longer than normal. Two other digits of unequal length were inserted along the ulnar border of the limb.

Exp. 1007, operation 27 May. Five weeks after the operation a small stump had developed, the distal end of which was curved downwards (Pl. 4, fig. 3).

Embryos operated after method 2 (*superficial implantation*)

Out of ten operated embryos, three survived:

Exp. 983, operated 19 May. The left limb showed two digits 5½ weeks after the operation. The development of the third digit was suppressed and in its place the implanted ectoderm was visible. The limb was normal in length (Pl. 4, fig. 4).

Exp. 999, operated 23 May. Five weeks after the operation a limb of normal length had developed, showing a deformed distal end which was formed by the implanted ectoderm and three deformed digits. Two of these consisted of small knob-like elevations, one of which had grown to an abnormal length.

Exp. 1009, operated 26 May. Four weeks after the operation a limb of normal length had developed, the distal end of which was bifurcated and showed two finger-like structures which were longer than normal.

Embryos operated after method 3 (*implantation along a vertical line*)

Out of thirty-one operated embryos five survived:

Exp. 993, operated 22 May. Four weeks after the operation the operated limb consisted of a short pointed stump, the proximal end of which showed some oedema, the distal end was formed by the implanted ectoderm.

Exp. 1000, operated 23 May. Five weeks after the operation the implanted ectoderm was attached along the whole length of the radial border of the limb. The distal end of the limb showed three finger-like elevations. The digits of the unoperated limb were fully developed. The total length of the operated limb was half the length of the normal limb (Pl. 4, fig. 5).

Exp. 1001, operated 24 May. Five weeks after the operation the implanted ectoderm was attached along the whole length of the radial border of the operated right limb. The pigmentation of the implanted ectoderm contrasted with that of the normal part of the limb which was half the length of the normal limb. A pointed distal end replaced the digits (Pl. 4, fig. 6).

Exp. 1002, operated 24 May. Four weeks after the operation the implanted ectoderm was attached to the side of the body of the larva 1 mm. distant from the root of the limb. The limb itself was normal in size and shape.

Exp. 1005, operated 25 May. Nineteen days after the operation the implanted ectoderm was attached to the dorsum of the proximal part of the limb. The distal end of the operated limb showed normal digits. The limb was stunted and there were no movements visible in the shoulder joint.

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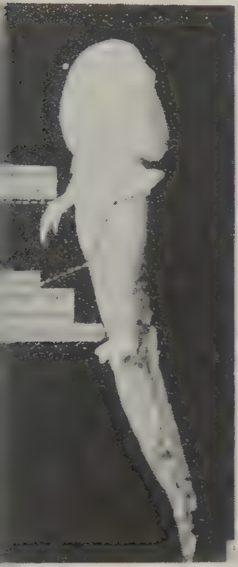
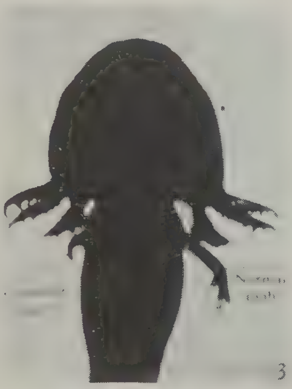
* The case represents an experimentally produced phocomelias and it can be compared with a case of phocomelias in a human newborn (Pl. 5) showing an abnormal shortness of the parts of the upper limb at which small finger-like processes are attached.





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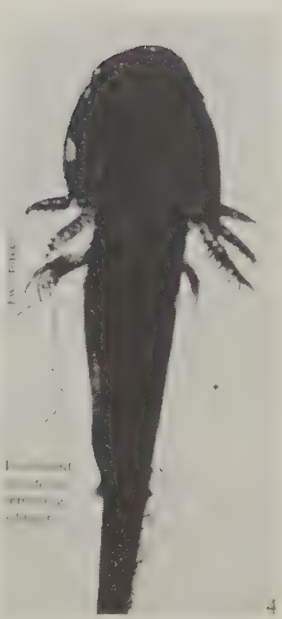

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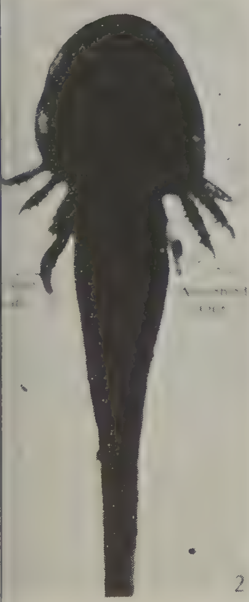

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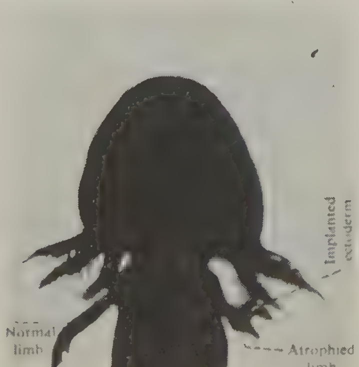

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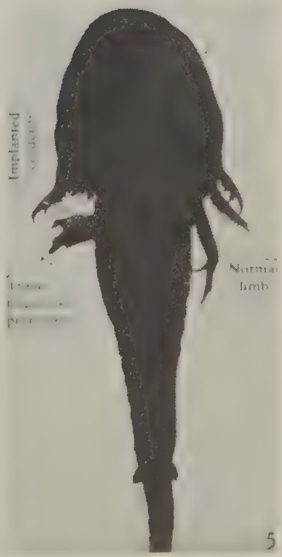

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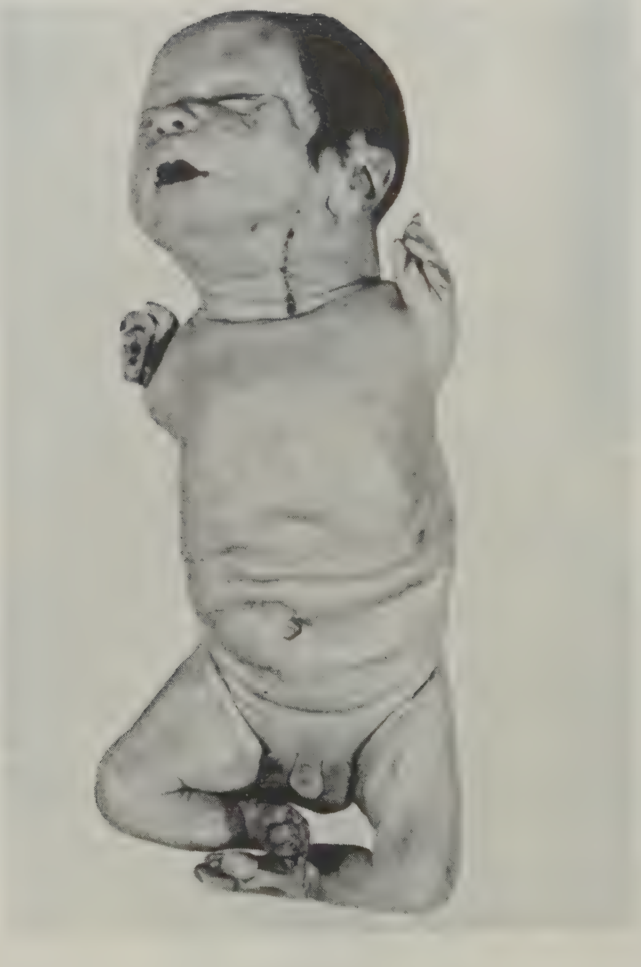
BRANDT—EXPERIMENTAL PRODUCTION OF ATROPHIED AND PARTLY DEFICIENT LIMBS (PHOCOMELIAS) IN THE AXOLOTL EMBRYO (pp. 117–119)



BRANDT—EXPERIMENTAL PRODUCTION OF ATROPHIED AND PARTLY DEFICIENT LIMB (PHOCOMELIAS) IN THE AXOLOTL EMBRYO (pp. 117–119)

SUMMARY

1. The results obtained indicate the development of some types of atrophied and locally deficient limbs as related to the method applied.

2. The larvae operated after method 3 (implantation along a vertical line) show the implanted ectoderm still attached along the radial border of the operated limbs (figs. 5 and 6). The length of these limbs is half as long as normal and the distal ends show rudiments of fingers, the number of which is reduced.

3. The length of the limbs, the primordia of which were operated after method 2 (superficial implantation), was always normal, but the digits of those limbs are either deformed (case 999) or they show the implanted ectoderm replacing a digit (case 983) or they possess an abnormal number of digits or some of the digits are abnormally long (case 1009).

4. All cases developing after application of method 1 (deep implantation) showed abnormally short and atrophied limbs. The type of deformity developing after this method is the so-called phocomelias. In the phocomelias the proximal parts of the limb are essentially reduced in length and the distal parts show normal or slightly reduced lengths of the digits.

5. Phocomelias is not uncommon in the human newborn and it is considered as a deformity developing on a genetical basis. It has not yet been proved that phocomelias can be produced experimentally by non-genetical methods. We can conclude from the experimental method applied that the ectoderm implanted into the deep area of the embryonic primordium of the limb bud acts as a factor preventing the normal efficiency of developmental potencies.

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EXPLANATION OF PLATES

Plate 4

Fig. 1. Exp. 963. Larva 26 days after the operation. The proximal part of the operated left limb is absent. Distal part of the limb only present, consisting of some finger-like elevations. Operated after method 1. The case represents an experimentally produced phocomelias. Compare the figure with Pl. 2 showing the human phocomelias.

Fig. 2. Exp. 975. Larva 6 weeks after the operation. The operated right limb is reduced in length and it shows edema in its proximal part. The fingers are absent and the distal end of the limb is pointed. Operated after method 1.

Fig. 3. Exp. 1007. Larva 5 weeks after the operation. The operated left limb consists of a pointed stump, and is essentially reduced in length. Operated after method 1.

Fig. 4. Exp. 983. Larva 5½ weeks after the operation. The operated left limb is normal in length and possesses two normal digits, the place of a third digit is taken by the implanted ectoderm. Operated after method 2.

Fig. 5. Exp. 1000. Larva 5 weeks after the operation. The implanted ectoderm is attached along the whole length of the radial border of the limb. The total length of the limb is half as long as the normal left limb. The distal end of the limb shows three short digits. Operated after method 3.

Fig. 6. Exp. 1001. Larva 5 weeks after the operation. The total length of the operated left limb is half as long as the normal right limb and shows three small finger-like elevations at its distal end. The implanted ectoderm is attached to the whole length of the radial border of the limb. Operated after method 3.

Plate 5

Phocomelias in the human newborn. The proximal parts of the upper limb are absent. The distal parts consist of some finger-like processes (compare with Pl. 1, fig. 1 showing the Axolotl). Specimen from the Department of Anatomy, University of Birmingham.

The photographs were made by the technician of the Department, Mr W. J. Pardoe.

ABSORPTION OF VOLATILE ACIDS FROM THE ALIMENTARY TRACT OF THE SHEEP AND OTHER ANIMALS

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(With 3 Text-figures)

It has long been known that volatile fatty acids are among the chief non-gaseous products of bacterial breakdown of carbohydrate in the rumen; however, in spite of the fact that the ruminant apparently subsists to a large extent upon the carbohydrates which are so broken down, investigators have been loath to admit the value of these to the animal. Instead, therefore, of following up the subsequent fate of the volatile acids they have concentrated more upon searching for unsuspected bacterial products which might make possible an explanation of ruminant carbohydrate metabolism more in line with the better known processes of absorption of sugars in non-ruminant animals.

Observations of the fate of the volatile acids have been few. Wilsing (1885) calculated, on the basis of *in vitro* experiments, that 157 g. of volatile acids would be produced by rumen fermentation from 1·5 kg. of hay which he fed to a goat. Of this only 2–5 g. was excreted in the urine and faeces. Phillipson & McAnally (1942) showed that while the concentration of volatile acids in the rumen ingesta is high, that in the abomasal ingesta is very low. They suggested that absorption of the acids in question from the rumen must occur. It has now been possible to show that volatile acids are in fact absorbed into the blood coming from the rumen, that this organ, together with the reticulum, omasum and caecum are the only parts of the alimentary tract from which any considerable absorption occurs and that the amount of volatile acid absorbed is sufficient to supply an appreciable part of the energy requirements of the animal.

I. ABSORPTION OF VOLATILE ACIDS FROM THE ALIMENTARY TRACT OF THE SHEEP, THE PIG, THE RABBIT AND THE PONY

Methods

Operative technique

In all experiments except that performed upon a pony, a solution of nembutal (60 mg. in 1 c.c.), given by slow intravenous injection, was used for an anaesthetic.

Sheep. The animal was laid on its left side, the abdomen was opened on the right side by a longitudinal incision extending backwards for 8–12 in. from the posterior border of the last rib and 4–6 in. below the transverse process of the lumbar vertebrae. A second incision from the anterior extremity of the previous one was made, parallel to the last rib, for 4–6 in. towards the mid line.

The abdominal viscera were covered by warm saline pads when it was necessary to withdraw them from the abdominal cavity, as was the case with the caecum, small intestine and abomasum.

Blood from the rumen was drawn, unless otherwise stated, from the posterior longitudinal rumen vein; blood from the reticulum was drawn from the two veins draining the right side of that organ, while a special procedure had to be adopted to obtain blood draining the omasum owing to the small size of the vessels. The epiploic vessels from the abomasum run over the greater curvature of the omasum and receive vessels draining that organ so that the blood of the epiploic vessel carries blood from both organs. In order to obtain omasal blood only, small bulldogs were placed on the epiploic vessels at either extremity of the omasum so that blood subsequently drawn from the isolated area came from the omasum only. No difficulty was experienced in obtaining blood from the abomasum, small intestine or caecum.

Pig. The vessels draining the alimentary viscera are small, and blood samples can be collected with ease only from the large vessels into which they drain at the root of the mesentery. Blood from the stomach was drawn from the left epiploic vein coursing along the greater curvature, but blood from the small intestine and colon was obtained from the large vessels at the base of the mesentery; with the colon it was necessary to separate gently the loops of the spiral in which it is arranged in order to expose the root of the mesentery.

Rabbit. The size of the animal limited the amount of blood that could be withdrawn without seriously upsetting the blood pressure. For this reason, in the first two experiments 5 c.c. samples only were taken, although 10 c.c. samples were taken in the second two experiments as in the experiments upon other animals. No difficulty was experienced in sampling.

Pony. We are indebted to Prof. E. D. Adrian for kindly permitting us to obtain from a pony peripheral blood, and blood draining from the small intestine and colon.

The use of a needle bent into a hook was found especially useful in obtaining blood from vessels that were not easily accessible or which were draining in a direction opposite to the only possible approach to them.

In all except the first few experiments the condition of the animal was gauged by measuring the blood pressure in the carotid artery.

Chemical technique

The separation of the volatile acids from the blood was accomplished by means of the technique described by McAnally (1944).

RESULTS

In the sheep numerous results show that the excess of volatile acid in the blood draining the rumen over that in the arterial blood is always considerable. Blood draining the reticulum contained much the same concentration of volatile acids as blood from the rumen. Absorption from the omasum, though much less than from the rumen or reticulum, is quantitatively significant. As might be expected from Phillipson & McAnally's (1942) previous results on the concentration of the acids in the abomasal ingesta, absorption from the abomasum appears to be nil; this is also the case with the small intestine; however, there is again absorption from the caecum where bacteria are again active and therefore a renewed production of volatile acid takes place.

Table 1. *Sheep*

Exp.	c.c. $N/100$ volatile acid per 100 c.c.* of blood taken from							
	Carotid artery	Jugular vein	Rumen vein	Reticulum vein	Omasum vein	Abomasum vein	Small intestine vein	Caecum vein
1	5	0	38	—	—	1	1	18
2	2	1	31	—	—	—	2	11
3	—	6	52	40	15	9	—	—
4	—	—	17	28	23	2	—	—
5	4	5	21	—	—	—	4	19
6	4	5	22	—	—	—	4	20
7	2	4	30	—	5	1	1	15
8	4	4	20	—	—	2	—	—
9	—	—	75	—	15	—	—	—
10	—	—	41	—	—	—	—	21
11	10	—	45	40	27	7	8	22
12	13	12	35	—	—	—	—	33
13	—	—	55	—	26	—	—	40
14	—	—	18	—	20	—	—	—

* Maximum error ± 4 c.c. $N/100$ %.

Table 2. *Pig*

Exp.	c.c. $N/100$ volatile acid per 100 c.c. of blood taken from						
	Carotid artery	Jugular vein	Stomach vein	Small intestine vein	Caecum vein	Colon vein	Portal vein
1	5	—	—	22	—	45	—
2	4	5	11	6	21	—	7
3	—	5	—	11	—	31	14
4	—	2	3	5	—	13	7

The chief sites of absorption in the pig are the caecum and colon; however, there is apparently some absorption from the stomach and small intestine. The possibility of there being some fermented material in the food was not excluded except in Exp. 3, where the concentration of volatile acid in the food was found to be only one-tenth of that in the stomach contents. It seems probable that even though the breakdown of the more readily fermentable constituents of the diet starts in the less acid cardiac end of the stomach and in the small intestine, conditions are not optimal until the caecum and colon are reached.

The results in the third table indicate that there is absorption from the caecum of the rabbit.

Absorption from the small intestine was not significant.

Table 3. *Rabbit*

Exp.	c.c. *N*/100 volatile acid per 100 c.c. of blood taken from				
	Jugular vein	Carotid artery	Small intestine vein	Caecum vein	Portal vein
1	0	0	0	11	—
2	—	—	—	30	10
3	—	8	6	28	8
4	—	3	9	25	—

Samples taken from a single pony showed that there is absorption of volatile acids from the colon of this species also.

Table 4. *Pony*

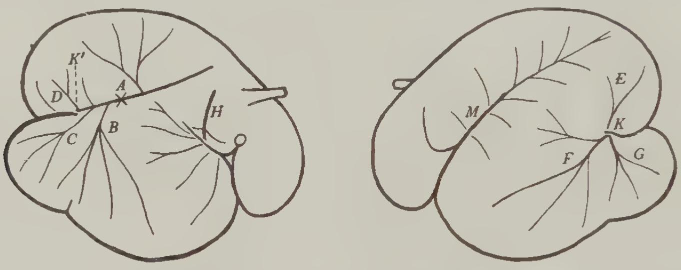
c.c. *N*/100 volatile acid per 100 c.c. of blood taken from		
Carotid artery	Small intestine vein	Colon vein
7	5	44

Samples of blood were taken from a number of different vessels which drain the rumen of the sheep. The position of the various vessels is shown in Fig. 1 and the concentration of volatile acids in the blood taken from them is given in Table 5.

Table 5

No.	Vein (see Fig. 1)							
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
1	52	15	6	26	71	46	—	—
2	17	8	10	—	—	29	11	51
3	41	—	—	—	—	—	—	50
4	45	—	—	—	—	—	—	88
5	55	—	—	—	—	—	—	109
6	18	—	—	—	—	—	—	65

The ventral sac of the rumen lay uppermost in the experiment as the sheep was lying on its side; here therefore the gas collected. Comparing the figure and table, clearly the vessels which drain the portion exposed to gas carry little volatile acid while those which drain the lower parts in which the ingesta are lying carry much. The anterior vein contains blood which carries the greatest concentration of volatile acids.



Right surface

Left surface

Fig. 1. Points from which blood was collected. The left surface (veins *E*, *F* and *G*) faced downwards in the experiment and the right surface (*B*, *C*, *D* and *H*) faced upwards. The vessel containing blood from *E*, *F* and *G* goes (as the animal was lying) up the posterior invagination between the dorsal and ventral sacs (*K*–*K′*) and therefore drains into *A*. *H* similarly receives most of its blood from the area drained by *M* and that adjacent to the groove up which it runs.

II. NATURE OF THE ACIDS ABSORBED

Having obtained evidence of the absorption of volatile acids from the alimentary canal, clearly the next step was to determine precisely what these acids were and in what proportion they occurred. For this purpose a comparison was made between the volatile acids in the blood leaving the rumen and those in the rumen content. Similar estimations were also made in respect of the contents of the caecums of two sheep and one pig. One pony was also studied but in this case the blood only was analysed, not the contents of the intestine.

The following procedure was adopted:

(1) *The blood.* Fifty c.c. of blood was taken from the posterior vein of the rumen, or the appropriate vein draining the portion of the alimentary canal under observation, and the separation of the mixed acids was performed in exactly the same way as for the smaller samples (McAnally, 1943).

(2) *The rumen content.* A sample of ingesta was taken from the rumen at the same time that the blood sample was obtained. It was filtered through muslin and to the filtrate an equal volume of acid magnesium sulphate precipitant (McAnally, 1943) was added. The precipitate was filtered off and the mixture of acids was separated from the filtrate by steam distillation as from the blood.

(3) *Other portions of the alimentary canal in the sheep and other animals.* The procedure was as in the case of the rumen.

For the determination of the nature and quantities of the various acids in these mixtures we have used the second distillation of Friedemann's method (1938). The exact details are given in the appendix. The method, however, depends upon the following principle. If the whole, in this case 200 c.c., of the solution containing the acids be distilled, the acids of higher molecular weight will come over more rapidly than those of lower molecular weight and therefore if the distillate be collected in successive fractions the earlier fractions will contain relatively more of the heavier acids.

Taking butyric, propionic and acetic acids; were pure butyric present, 37 % of it would come over in the first 25 c.c., 27 % (64–37) in the second 25 c.c. and so on according to the curve given in Fig. 2. Were the acid pure propionic acid, only 20·5 % would come over in the first 25 c.c. of distillate, 20 % in the second and so on—in the case of pure acetic acid 10 % comes over in the first, 10·5 in the second and so on.

Within the limits of experimental procedure these proportions are unaltered by the rate of distillation

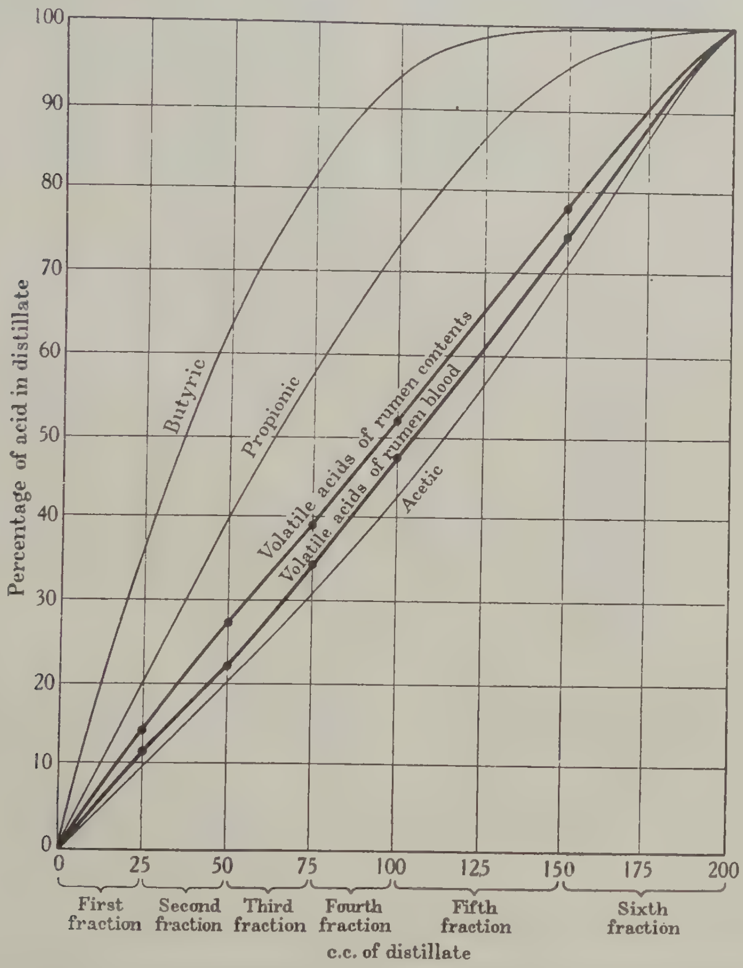


Fig. 2. Rate of 'Friedemann distillation' of volatile acids from the rumen contents and the blood respectively on a background of curves obtained from distillation of pure acids.

and in a mixture of the acids the rate at which each distils over is independent of the presence of the others. When, therefore, as in the case of the distillate from the blood from the rumen the rate of distillation of the total acids present is almost coincident with but a little more rapid than that of acetic acid the inferences may be drawn: (1) that the great preponderance of acid present is acetic, (2) that there is some acid of higher molecular weight than acetic.

Further, when from the rumen content the earlier samples of distillate are richer in volatile acid than in the case of those from the blood, it may be inferred that there is less acetic relatively to acids of higher molecular weight in the rumen content than in the blood.

Table 6

Animal	c.c. distillate	% of acid in successive fractions of distillate					
		25	25	25	25	50	50
—	Pure acetic*	10	10·5	11	11·5	28·5	28·5
—	Pure propionic*	20·5	20	18	15·5	21·5	4·5
—	Pure butyric*	37	27	18·5	11·5	6	0
Sheep A	Rumen contents†	17	15	14	12	23	19
	Rumen blood†	14	13	11·5	11	25	25·5
Sheep B	Rumen contents	17	13	14	13	23	20
	Rumen blood	26·5		12	10·5	24	27
Sheep C	Rumen contents*	14	13·5	12	12·5	25·5	22·5
	Rumen blood*	12	10·5	12·5	13	27	25
Sheep D	Rumen contents	17	14·5	13	12·5	22·5	20·5
	Rumen blood	15	10·5	12	12	25·5	25
Sheep	Caecal contents	13	12	12	12	26	25
Sheep	Caecal contents	14	14	13	10	20	29
Pig	Caecal contents†	16·5	15	14	12·5	23	19
Pony	Colon blood†	15·5	13	12	12	24	23·5

* Plotted in Fig. 2.

† Much more precise results as to the quantitative distribution of the acids have been obtained by means of a chromatographic method to be published shortly by Dr S. R. Elsden. He has very kindly estimated the amounts of the acids in distillates of the materials indicated and has been able to confirm the conclusions which we have drawn.

Finally, the figures in Table 6 show that the products of fermentation in the caecum of the sheep and the pig and in the colon of the pony are similar to those in the rumen of the sheep.

III. ABSORPTION OF THE INDIVIDUAL ACIDS INTRODUCED INTO AN EMPTY RUMEN

The conclusion was drawn from the results obtained in the previous section that blood draining the rumen contains a higher proportion of acetic acid to acids of higher molecular weight than do the contents of the rumen. There seemed thus to be an indication that acetic is more rapidly absorbed than are the higher acids. The following experiments were designed to see whether this was so.

Two 5 months old ram lambs and one adult wether were fitted each with two rumen canulae, one being in the usual position, in the flank, as already described by Phillipson & Innes (1939), and the other inserted in the ventral sac and brought out 2 in. to the left of the mid-line in the umbilical region. It has been found possible to wash out completely the rumen contents of such an animal if it has previously been fed on a finely divided fodder such as bran and oats. The rumen was filled with 2 l. of water and the animal then anaesthetized, the abdomen opened, and blood taken from the posterior rumen vein. 25 g. of sodium acetate in 2 l. of water at body temperature were then introduced into the rumen through the canula, and a sample of rumen blood was again taken. The acetate was washed out and replaced by warm water and blood again taken. The same process was repeated giving a dose of 25 g. of sodium propionate in 2 l. of water, followed by water, then 25 g. of sodium butyrate in 2 l. of water. In Exps. 2 and 3 as a control measure in case any abnormal condition of the rumen epithelium might have been induced by the experimental procedure, after measurement with water in the rumen the sodium acetate dose was again given.

Examination of Table 7 will show that while blood draining from the empty rumen or the rumen filled with water contains no detectable volatile acid, no more than 5 min. after filling the rumen with sodium acetate solution the concentration of the acids in the blood has risen very appreciably. The absorption of propionate after the same time is not so great as with acetate, while butyrate appears to be little absorbed in this space of time. Samples of peripheral blood were also taken in Exp. 1 and there was some indication that, even after so short a time, the concentration of volatile acid in the peripheral blood was influenced by the contents of the rumen, but the amounts of acid titrated in these samples were so small that the differences were not outside the range of experimental error. Acetate was again absorbed when given after the other two salts had been tested but the concentration in the blood was less than that found previously. Thus, though comparison of the butyrate absorption with the second acetate absorption clearly indicates that the former is very much less rapidly absorbed, the relative rates of absorption of propionate and acetate were not established with such certainty.

Table 7

Exp.	Blood sample taken from	c.c. of $N/100$ volatile acid per 100 c.c. of blood							
		Blood sample taken 5 min. in Exps. 1 and 2; 15 min. in Exp. 3 after introduction into the empty rumen of							
		2 l. water	25 g. Na acetate in 2 l. water	2 l. water	25 g. Na propionate in 2 l. water	2 l. water	25 g. Na butyrate in 2 l water	2 l. water	25 g. Na acetate in 2 l. water
1	Carotid artery	0	3	0	2	0	3	—	—
	Jugular vein	0	2	0	5	0	0	—	—
	Rumen vein	0	32	6	20	4	6	—	—
2	Rumen vein	1	14	3	10	2	2	0	11
3	Rumen vein	2	72	21	58	3	8	2	51

In order, therefore, to test this latter point a further experiment was performed. An eighteen months old wether was anaesthetized and a single canula was inserted into the ventral sac of the rumen; the wound was closed around the neck of the canula. It was found possible to empty the rumen and wash it clean with warm water through this single canula (the animal was fed upon bran and oats for about a week before the experiment and starved for the last 24 hr.). The rumen was filled with 2 l. of warm water. The oesophagus was tied in the neck and the abdomen then opened on the right side in the usual manner. A ligature was then passed round the abomasum excluding the epiploic vessels immediately below the omasum so that no escape from the rumen was possible.

The previous experiments were then repeated with the following differences: (1) the order of the doses was water, propionate, water, acetate; butyrate being omitted; (2) the acetate was allowed to remain in the rumen for 90 min., samples of blood being withdrawn at intervals; (3) equimolecular solutions were used.

The results shown in Table 8 demonstrate clearly that the rate of absorption of propionate is lower than that of acetate. At the end of the experiment the remainder of the dose containing acetate was carefully collected in addition to the washings from the rumen, so that the amount of acetate absorbed during the 90 min. interval could be estimated. On post-mortem examination the rumen and reticulum were clean and empty but some ingesta were present in the omasum. As it was not impossible that some of the acetate solution had passed into the latter organ the ingesta were washed out and collected, the quantity of volatile acid found here was, however, negligible.

The total loss of sodium acetate during the 90 min. interval was 10 g. If this rate of absorption is calculated on the basis of grams of acetic acid per hour, it establishes the fact that the rumen and reticulum together can absorb at least 5 g. of acetic acid in this time. This quantity is rather greater than the amounts which we have calculated to be absorbed from the rumen containing normal ingesta. It is clear therefore that the calculated absorption is not outside the range of absorption of which the rumen is capable. It is interesting that the reduction of volume of the liquid in the rumen during the 90 min. interval was only 150 c.c.

Table 8

Dose introduced into the rumen	Time after introduction of dose when blood taken min.	c.c. $N/100$ volatile acid per 100 c.c. of blood
2 l. of water	5	0
2 l. of water containing 35 g. of anhydrous sodium propionate	5	31
Do.	15	30
2 l. of water	5	3
2 l. of water containing 25 g. of anhydrous sodium acetate	5	49
Do.	15	55
Do.	30	72
Do.	60	66
Do.	90	56

The fact that the rate of absorption of propionate is less than that of acetic both at the start of an experiment, when physiological conditions are optimal and at the end when the conditions are less good (as shown by a fall in blood pressure) indicates clearly that the acid of higher molecular weight is less rapidly absorbed.

IV. THE RATE OF ABSORPTION OF VOLATILE ACIDS FROM THE RUMEN

In order to determine the quantity of volatile acid absorbed in a given time from the rumen it was necessary to measure the rate at which blood flows from it as well as the concentration of volatile acids carried in this blood. It was found to be impossible to gain access to the vessel into which the tributary veins from the rumen flowed, neither was it possible to expose any vessel draining the rumen, except the posterior longitudinal vein, sufficiently to measure the rate of blood flow through it. It was decided, therefore, to measure the blood flow in the posterior vessel and from this to calculate the amount of blood draining both the rumen and reticulum in a given time on the assumption that the blood flow is directly proportional to the area drained.

Experimental procedure

The preliminary operative procedures have already been described. The blood pressure was taken from the carotid artery before and after opening the abdomen. A short length of the posterior vein in the vicinity of *A*, Fig. 1, was dissected free from the visceral peritoneum and a ligature was passed beneath it. A short needle with a bore of 1·5–2 mm., to which was attached a short length of rubber tubing of approximately the same bore, was thrust into the vessel against the blood flow and the ligature was immediately tied so that the shaft of the needle was held in the vessel. The knot of the ligature was given a single hitch only so that it could be pulled tight immediately on the withdrawal of the needle from the vein. The needle and rubber tube were held horizontally and the end of the tubing was turned downwards into a measuring cylinder. The cylinder was changed every half minute, the time being taken by stop watch, and the half minute output of the vessel was thus measured over a period of from 2½ to 3 min. At the end of this time the needle was withdrawn from the vein and the ligature pulled tight and the carotid blood pressure was again taken in order to see if any serious fall had occurred through loss of blood. In one experiment the blood pressure tracing was taken throughout the period of collection and is shown in Fig. 3. An average figure for the half-minute readings was taken and doubled to give the output of the vessel per minute.

An important precaution in performing these manipulations was to ensure that the flow of blood from the vein was unimpeded in any way. It was essential to hold the needle firmly so that there was no danger of the walls of the vessel forming a valve over the oblique opening of the needle.

The course of one experiment is given below to illustrate the procedures described:

Sheep 25.

Blood pressure at start of experiment = 140 mm. Hg

Abdomen opened and posterior vein of the rumen exposed

Blood pressure = 128 mm. Hg

Blood flow of posterior vein measured

Blood collected during successive 30 sec. intervals:
42·0 c.c., 44·9 c.c., 40·5 c.c., 39·5 c.c., 41·0 c.c.

Blood pressure at end of experiment = 116 mm. Hg

Total blood collected in 2½ min. = 207 c.c.

∴ Blood flow per minute = 83 c.c.

Area of rumen and reticulum drained* = 31 %

Concentration of volatile acid in blood calculated = 15 c.c. 0·01 *N* %

Calculations

Blood flow from whole of rumen and reticulum = 268 c.c. per min.

Volatile acid drained from these organs = 40·2 c.c. 0·01 *N* per min.

Volatile acid drained per minute in g. acetic acid = 0·024 g.

Volatile acid in g. acetic acid drained per hour = 1·44 g.

In most of the experiments the orifice of the rubber tube from which the blood was collected was held at the level of the vein; in sheep 29, however, the actual venous pressure was measured by a method devised by one of us for another purpose, namely, the insertion of a hypodermic needle into the vein; to the needle is attached a piece of translucent rubber (bicycle valve) tubing containing Ringer's solution, the solution runs into the vein till it falls to the level of the venous pressure. The meniscus can be seen through the wall and its height measured with a rule. The puncture in the vein causes no subsequent trouble. The pressure as measured in this experiment was 16·4 mm. Hg (mean of 16·1 and 16·7). During the subsequent collection of the blood samples the orifice of the collecting tubule was held at the level to which the meniscus had fallen. The venous pressure was thus taken into account and the resulting picture of blood flow was not significantly different from that in other experiments. The consecutive flows were: (1) 46, (2) 46, (3) 46, (4) 44 c.c. per 30 sec.

The blood pressure is given in Fig. 3.

At the end of the experiment the oesophagus and duodenum were ligated and the whole stomach was removed from the carcass. The area of the rumen drained by the posterior vein to the point of puncture

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* The method of estimating this is given later.

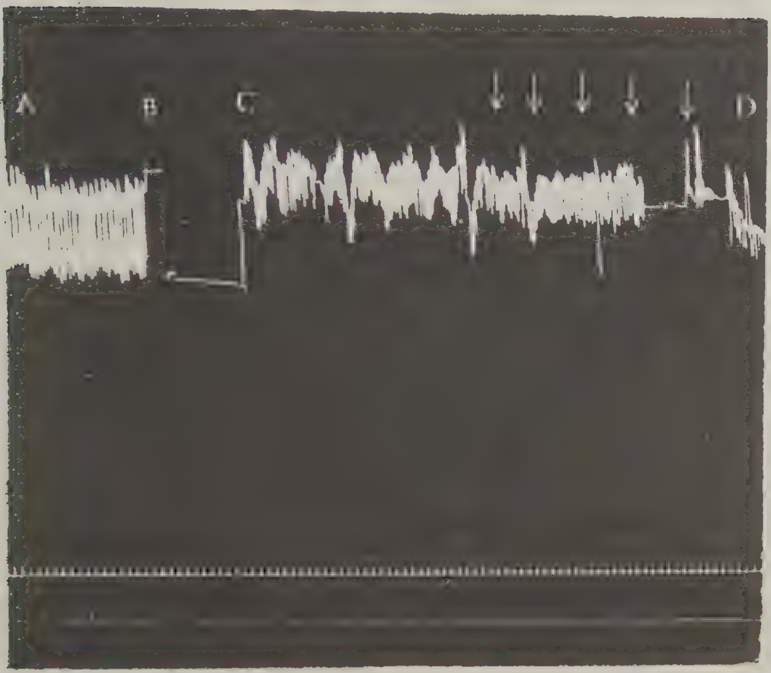

Fig. 3. Tracing of carotid pressure from sheep 29. Lowest line = base line. Time interval = 5 sec. *A–B*, tracing before opening of abdomen. *C–D*, during actual period of collection. The intervals between the arrows represent approximately those in which four 30 sec. samples were taken. (At the fourth sample a small clot was dislodged from the canula.)

was marked off by a line made with a solution of hexamethyl violet. This area was separated from the remainder of the organ and cut into pieces so that it could be spread flat upon a table and its area measured. The surface area of the remainder of the rumen and of the reticulum were similarly measured and the proportion of the whole represented by the area drained by the posterior vein was calculated.

The area drained was found to vary between 31 and 43 % of the total surface area of the rumen and reticulum, the average figure from fourteen measurements being 37 %. In each calculation the individual measurement for the sheep concerned was used in estimating the rate of absorption per hour.

RESULTS

Only those results are recorded in which:

(1) The figures for the output of the posterior vessel were reasonably constant and not steadily falling.

(2) The blood pressure did not fall below 80 mm. Hg during the experiment.

(3) The sheep was found to be normal on post-mortem examination.

The measurements of blood pressure made during the ten experiments given in Tables 10 and 11 were as follows:

Table 9

Sheep	Blood pressure in mm. Hg at start of experiment	After abdomen was opened mm. Hg	After blood flow was measured mm. Hg
13	136	—	100
15	128	—	106
16	144	—	126
18	120	—	90
19	120	—	112
23	86	134	80
24	120	116	110
25	140	128	116
26	122	120	116
27	149	—	129
29	See Fig. 3		

The results are shown in Tables 10 and 11. Those in the former table were obtained from sheep which were taken from pasture in October and early November, while those shown in the latter table were obtained from sheep taken from grass in April and May. Of the five experiments done in the spring three were done at midnight, Nos. 23, 24 and 27, and two were done at mid-day, Nos. 25 and 26. This was done as previous results (Phillipson, 1942) showed that the maximum concentration of volatile acid in the ingesta in the rumen occurred at midnight, and it was thought that for this reason absorption would be greater at night than during the day. The results, however, show no significant difference between the quantity of volatile acid absorbed per hour in the daytime and at night.

Table 10

Sheep	Volatile acid of rumen contents as g. acetic acid per 100 g. dry matter	Blood flow per minute from posterior vein c.c.	Concentration of volatile acid in c.c. 0·01 *N* per 100 c.c. rumen blood	g. volatile acid absorbed per hour
13	6·92	87	43	3·9
15	6·09	59	55	3·7
16	6·79	107	35	3·5
18	6·04	126	44	5·3
19	6·50	67	35	2·6
29	6·26	92	26	2·3

Table 11

Sheep	Volatile acid of rumen contents as g. acetic acid per 100 g. dry matter	Blood flow per minute from posterior vein c.c.	Concentration of volatile acid in c.c. 0·01 *N* per 100 c.c. rumen blood	g. volatile acid absorbed per hour
23 night	9·75	88	39	3·7
24 ,,	8·07	38	29	0·9
25 day	7·64	83	15	1·4
26 ,,	7·93	82	22	1·6
27 night	6·84	67	40	2·5

A tendency for absorption to be greater in the autumn than in the spring is apparent in the tables although there appears to be no reason why this should be so and in view of the small numbers of sheep employed no significance can be attached to this result.

The variation in the output per minute of the posterior vein of different sheep is related to the size of vessel. Thus in sheep in which the flow was above 100 c.c./min. the vessel was large while the vessel was small in sheep 24 in which the lowest figure, namely, 38 c.c./min., was recorded. The size of vessel cannot be correlated with either the weight of the animal or the weight of the ingesta.

The variation in the concentration of the volatile acid in the blood draining the rumen does not exceed the range found in previous experiments.

It is of interest to see whether or not the concentration of volatile acid in the ingesta of the rumen has any relation to the quantity of volatile acid absorbed. The concentration of volatile acid is expressed as gm. acetic acid per 100 g. dry matter in column 2 of Tables 10 and 11. It can be seen that the concentration in the autumn sheep was remarkably regular and so cannot account for the variation in the rate of absorption. In the spring sheep the average concentration was higher, yet in spite of this the rate of absorption in three animals was low. The only conclusion that can be drawn is that the rate of absorption does not depend upon the concentration of volatile acid in the ingesta.

V. DISCUSSION

The results obtained here fully confirm the previous inference that absorption of volatile acids occurs from the rumen. The fact that absorption in the omasum is less although still significant suggests that this organ, in addition to giving the solid ingesta a final comminution before it enters the abomasum, also removes the remainder of the volatile acids from the ingesta so that the material entering the abomasum is practically free from these acids.

The view that the omasum acts as a sponge was put forward by Favilli (1937); it is well known that the ingesta in the omasum has a higher percentage of dry matter than that of the rumen and as in addition volatile acids are absorbed from this organ the view put forward by Favilli has some support.

The fact that volatile acids reappear in the blood draining the caecum is a clear indication that fermentation of carbohydrate again commences in the large gut. The recent work of Trautmann & Asher (1939, 1941), in which the fermentation of cellulose in the caecum of goats was investigated, does not support the view that fermentation of this substance is responsible for the acids absorbed from the caecum although their conclusions are open to criticism (McAnally & Phillipson, 1944). It is sufficient here to point out that the mixture of volatile acids present in the caecum is essentially the same as that present in the rumen, in the caecum of the pig and in the blood draining the colon of the horse in all of which organs fermentation of carbohydrate, of which cellulose is an important component, is known to occur. It must, however, be allowed that the products of fermentation of the individual carbohydrates in these organs are not known, consequently this mixture of volatile acids may be characteristic of fermentation of carbohydrates other than cellulose.

The rate of absorption of volatile acid from the rumen was previously given as varying from 2 to

4 g./hr. (McAnally & Phillipson, 1942); this range must now be extended to 0·9–5·3 g./hr.

These values must be regarded as minimal for the total absorption of volatile acid for the following reasons:

(1) The papillae of the rumen greatly increase the absorbing area and as those of the anterior region are larger than those of the posterior region (the area drained by the posterior vein) measurements of the surface area, such as have been made, must give a higher value for the portion of the rumen drained by the posterior vein than is actually the case.

(2) The anterior vessel of the rumen was found to carry a higher concentration of volatile acid in the blood than the posterior vessel.

(3) Volatile acids are absorbed from both the omasum and the large intestine and no account has been taken of this fact.

(4) Volatile acids may be absorbed by the lymphatic system.

It is important to bear these errors in mind, for if the total carbohydrate digested by a 50 kg. sheep is calculated in terms of total carbon digested, on the assumption that polyhexoses predominate, it is found that after a deduction of 15% for carbon lost as methane and carbon dioxide, the animal absorbs 264 g. carbon a day at maintenance level. If the highest rate of absorption, namely, 5 g. of volatile acid per hour calculated as acetic acid, is taken, then only 48 g. of carbon can be accounted for in this way, approximately one-sixth of the total requirements. If this figure, however, is accepted, it does represent a significant portion of the digestible carbohydrate and as such is of nutritional interest. This is especially so when it is recalled that the production of volatile acids is common to carbohydrate fermentation in the horse, pig and rabbit and presumably in all herbivorous animals and possibly in man; consequently the nutritional value of these acids warrants further investigation.

VI. SUMMARY

1. The concentration of volatile acids in the blood draining the rumen is considerably higher than that of peripheral blood in which little volatile acid, if any, is present.

2. Volatile acid in significant amount is present in blood draining the omasum and the caecum but is absent from blood draining the abomasum and small intestine.

3. Volatile acid is also found in significant concentration in blood draining the caecum or colon of the horse, pig and rabbit.

4. Distillation curves indicate that acetic is the predominant acid present in both the rumen contents and in the blood draining the rumen, but the proportion of acids of higher molecular weight than acetic is greater in the rumen contents than in the blood. The same type of distillation curve is found for material taken from the caecum of the sheep and the large intestine of the horse and pig.

5. The rate of absorption of the sodium salts of acetic, propionic and butyric acids, as judged by the concentrations found in the blood leaving the rumen, appears to be in the order of their molecular weights; acetate is rapidly absorbed, propionate less so, while butyrate is slowly absorbed.

6. The quantity of volatile acid calculated as acetic acid absorbed in an hour from the reticulum and rumen together is estimated to vary from 1 to 5 g., but this value must be regarded as minimal.

We wish to thank Mr H. Bowman and Mr D. Chilvers for valuable technical assistance.

APPENDIX

The distillate containing mixed volatile acids was made slightly alkaline and then evaporated down to somewhat less than 200 c.c. The solution was made up to exactly 200 c.c. and this, with 10 c.c. of acid mercuric sulphate solution and solid magnesium sulphate (the quantities are given in Friedemann's paper), was distilled. Four consecutive 25 c.c. fractions were collected and titrated against $N/100$ KOH, then a 50 c.c. fraction was similarly treated and finally distillation was continued until crystallization occurred in the flask; this final fraction of distillate thus obtained was about 50 c.c. in volume.

The amount of acids in the whole distillate was summed and the percentage of the whole in each fraction calculated.

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THE DETERMINATION OF TOTAL VOLATILE ACIDS IN BLOOD

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In order to study the absorption of volatile acids from the alimentary canal of the ruminant it was necessary to adopt a method of determining the concentration of these acids in the blood.

A method for the determination of volatile acids in various biological materials has been described by Friedemann (1938) which is said to be applicable to blood. The blood is added to dilute sulphuric acid, then sodium tungstate solution and solid magnesium sulphate are added and the whole is steam distilled. Particular attention is not paid to blood, however, and no comparative results or figures for recovery of added acids are given. Further, Friedemann suggests in a footnote (p. 167) that decomposition of labile substances leading to high results may occur when large samples of urine are distilled. In view of this observation it was thought probable that a similar error might arise in analyses of blood; experiments were therefore carried out to test whether there is any indication that the Friedemann procedure may lead to the distillation of volatile acids not originally present in the blood. 10 c.c. samples of bullock's blood were distilled exactly as described by Friedemann; some samples were distilled as fast as possible, however, while others were taken over more slowly. If no decomposition is occurring the time taken for the distillation should have no effect upon the volatile acid present in the 200 c.c. of distillate recommended by Friedemann as being sufficient for complete separation of the acids in 10 c.c. of blood. The following table indicates that more prolonged distillation results in higher answers, thus even when the distillation time is a minimum, it cannot be assumed that no decomposition has occurred.

Time taken for distillation min.	c.c. $N/100$ acid in 200 c.c. of distillate c.c.
28	0·99
35	0·94
51	1·00
59	1·70
60	1·76

It was therefore thought advisable to remove the protein precipitate before steam distilling. If the precipitate obtained by Friedemann's procedure was filtered off and the filtrate distilled a very poor recovery of volatile acids was obtained. A simpler precipitation method has, however, been found to give good recovery of acetic, propionic and butyric acids added to blood.

The method, like Friedemann's, is based upon the observation of Olmsted, Whitaker & Duden (1929) that high concentrations of magnesium sulphate greatly increase the rate of distillation of volatile acids. The essential steps in the method are precipitation and removal of proteins, acidification and steam distillation in the presence of magnesium sulphate.

Precipitation of the protein with a volume of saturated magnesium sulphate solution, containing sulphuric acid, equal to the volume of blood taken was at first practised; however, it was found that though added acetic acid was well recovered (90–100% recovery) butyric was less well recovered (70–80%) and propionic acid was intermediate. If the blood is considerably diluted with water before precipitation, however, recovery of all three acids is equally good. Furthermore, since by this means the blood is laked before precipitation, all volatile acid whether in plasma or corpuscles is estimated together.

REAGENTS REQUIRED

Saturated magnesium sulphate solution to which has been added 2·5% by volume of concentrated sulphuric acid. $N/100$ KOH.

METHOD

To one volume of oxalated blood four volumes of water are added and then five volumes of the acid magnesium sulphate solution. After standing a few minutes the precipitate is filtered off and a clear colourless filtrate is obtained. An aliquot of the filtrate (equivalent to one-tenth its volume of blood) is taken for the distillation. The apparatus recommended by Friedemann was used. The volume of the sample is rapidly reduced to one-half by adjusting the height of the burners under the steam generator and distilling flask and then steam is passed while the volume in the distilling flask is kept constant until 50 c.c. has been distilled over. If a

25 c.c. aliquot of filtrate is distilled the initial frothing can soon be controlled and therefore there is no difficulty in reducing the volume to one-half before much more than 25 c.c. of distillate has been taken over. The remaining 25 c.c. of distillate then seem sufficient to carry over the rest of the volatile acid in the sample. When a 50 c.c. aliquot is distilled the frothing is more troublesome unless a larger distilling flask is taken and the reduction in volume cannot be effected so quickly. It may then be necessary, particularly if much acetic acid is present, to collect a further 50 c.c. of distillate to complete the separation. The time taken for distillation does not appear to affect the results. The distillates are aerated for 3 min. with CO_2 free air and, while the aeration is continued, the acid in the distillate is titrated against $N/100$ KOH added from a burette whose tip passes through a rubber bung directly into the titrating flask.

RECOVERY OF ACIDS FROM BLOOD

c.c. of *N*/100 volatile acids in			
5 c.c. of blood	5 c.c. of blood to which 1·61 c.c. of acetic acid added	5 c.c. of blood to which 1·15 c.c. of propionic acid added	5 c.c. of blood to which 1·40 c.c. of butyric acid added
0·17 c.c.	1·66 c.c.	1·30 c.c.	1·49 c.c.

Recovery of acetic = 92·5 %.
Recovery of propionic = 98·3 %.
Recovery of butyric = 94·3 %.

A larger apparatus has been successfully employed for the separation of volatile acids from greater volumes of blood when further differentiation between the acids in the distillate is contemplated.

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THE ABSORPTION OF SODIUM ORTHO-IODO-HIPPURATE FROM THE RUMEN OF LAMBS

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(With Plates 6 and 7)

Previous work (Phillipson & McAnally, 1942) indicates that absorption from the rumen is not only possible, as was shown by Trautmann (1933), but is rapid enough to prevent passage to the abomasum of volatile acid present in the rumen, although these acids are present in the rumen in a high concentration. This evidence is remarkable, as the epithelium lining the rumen is stratified and squamous qualities not usually associated with membranes through which there is active absorption.

Direct evidence regarding the properties of the rumen as an organ of absorption was necessary, and the experiments described in this paper show that even a large molecule such as sodium ortho-iodo-hippurate can pass through the epithelium of the rumen in significant amounts.

METHOD

Six young lambs up to four months old were used, they were anaesthetized by intravenous injection of nembutal and the abdomen was opened in the mid-line. The abomasum was withdrawn and the thin muscular connexions between it and the rumen and reticulum were severed to allow a ligature to be passed, excluding the epiploic vessels, around the omasal-abomasal orifice, so that the rumen, reticulum and omasum, all of which are lined by stratified squamous epithelium, were isolated from the remainder of the alimentary tract. The abdomen was closed and the animal was taken to the Department of Anatomy and a plate was taken of the abdomen. Sodium ortho-iodo-hippurate was then administered by stomach tube at the rate of approximately 2 g./kg. body weight in watery solution, and its passage into the rumen was observed under the screen. Further exposures were made at suitable intervals after dosing and observations were continued up to 5 hr. Two of the lambs were used as controls, and the same procedure was adopted as with the animals dosed with the opaque salt except that the dose given was water. All animals were kept anaesthetized by further injections of nembutal throughout the course of the experiment.

At the end of 5 hr. the animals were killed, the bladder emptied and the quantity of urine measured. The presence or absence of iodine was demonstrated in the urine by ashing in the presence of excess caustic soda, liberation of iodine with nitrous acid and extraction with chloroform. Finally, the position and efficiency of the ligature at the omasal-abomasal orifice was tested to ensure that no passage of the opaque salt from the rumen to the abomasum had occurred.

RESULTS

Absorption of the opaque salt was demonstrated first by shadow thrown by the bladder and secondly by the presence of iodine in the urine. The details of each experiment were as follows:

Exp. 1. Ram lamb, 10 days old. Weight 3·8 kg. Dose 10 g. iodo-hippurate in 20 c.c. water. No shadow thrown by the bladder 1 hr. after dosing. Distinct shadow thrown 4½ hr. after dosing. Positive iodine reaction in urine.

Exp. 2. Ram lamb, 3 months old. Weight 23·7 kg. Dose 40 g. iodo-hippurate in 80 c.c. water. Bladder clearly visible 1½ hr. after dosing; shadow dense 3½ hr. after dosing. Bladder contained 189 c.c. urine and gave strong positive reaction for iodine.

Exp. 3. Ram lamb, 3 weeks old. Weight 6·35 kg. Dose 50 c.c. water. No shadow thrown by bladder at 25 min., 2 hr. 40 min. or at 5 hr. after dosing. Bladder contained 67 c.c. urine and gave negative reaction for iodine.

Exp. 4. Ewe lamb, 3 months old. Weight 25·7 kg. Dose 100 c.c. water. No shadow thrown by bladder until 5¼ hr. after dosing when a faint shadow was visible. Bladder contained 125 c.c. urine and gave negative reaction for iodine.

Exp. 5. Ram lamb, 3 months old. Weight 16·9 kg. Dose 35·3 g. iodo-hippurate in 106 c.c. water. Plain shadow thrown by bladder 5 hr. after dosing. Urine gave strong positive reaction for iodine.

Exp. 6. Ram lamb, 3 months old. Weight 18·7 kg. Dose 37 g. iodo-hippurate in 74 c.c. 1% sodium acetate. Bladder opaque 5 hr. after dosing. Bladder contained 72 c.c. urine and gave strong positive reaction for iodine.

The greatest absorption, as judged by the opacity of the bladder, occurred in Exp. 2 and the plates of this experiment are shown in Pl. 6 together with one of a control animal.

The plates obtained in Exp. 1 were not as clearly positive as in the other experiments, but the presence of iodine in the urine confirmed that absorption had taken place.

In order to test the stability of sodium ortho-iodo-hippurate during exposure to bacteria of the rumen, the salt was added to liquid rumen ingesta in a concentration comparable to that attained after dosage into the rumen and the material was incubated for 5 hr. at 37° C. At the end of this time no free iodine could be detected in the flask indicating that the benzene ring was not destroyed. This was further confirmed by the fact that iodine excreted in the urine was in organic combination.

Anatomy of the rumen in relation to absorption

The surface area of the reticulum and rumen combined is not large proportionally to the weight of ingesta contained in these organs. The presence of invaginations of the walls forming the muscular pillars, however, makes the absorbing surface larger than is apparent at a cursory glance. The surface area is, in addition, studded with numerous papillae; these are especially large in the anterior region of the rumen and greatly increase the surface area.

The epithelium of the rumen, when studied histologically, differs from that of the skin in the following ways (Pl. 7):

(1) The layer of cornified cells is thin and rarely exceeds a depth of more than three to four cells. This is due to the continual maceration to which the epithelium is subjected by the semi-liquid contents of the rumen and to the continual friction applied to the epithelium by the movement of the ingesta.

(2) The layer of granula cells, the stratum granulosum, although distinct in many places is by no means a continuous layer.

(3) No sebaceous glands are present, consequently there is no greasy protection to assist in preventing the passage of water through the epithelium.

The blood vessels of the rumen have been studied in four specimens in which the vessels were injected with gelatine carmine. The vascularity of the rumen in relation to the omasum and abomasum can be judged by reference to Pl. 7. The capillaries come into intimate relation with the deep cells of the malpighian layer and penetrate into the denticular spaces.

The omasum, of which a section through one of the laminae is shown, is similar to the rumen while the number of large vessels distributed through the laminae testifies to vascularity of the organ.

DISCUSSION

These experiments demonstrate conclusively that absorption of a molecule as large as sodium ortho-iodo-hippurate can occur through the epithelium of the rumen. The rate of absorption, however, is not rapid, for a considerable quantity of the drug was retained in the rumen at the end of the experimental period. In Exp. 2 an approximate estimation of the quantity of the drug excreted in the urine was made from the quantity of iodine recoverable and found to amount to one-sixth of the dose.

In comparing the epithelium of the rumen to that of the skin it should be pointed out that absorption through the skin is possible and the rapid absorption of boric acid (Kahlenberg, 1924, 1928), and a somewhat slower absorption of salicylic acid (Leslie-Roberts, 1928), has been demonstrated. These compounds, however, were the only ones absorbed of many that were tried. Luciani (1913) states that the removal of the sebum from the skin by ether allows the absorption of certain salts that are not otherwise absorbed.

The skin presents three barriers to prevent absorption. These are: first, the secretion of the sebaceous glands; secondly, the keratinized external layer of dead cells; and thirdly, the deep cells of the malpighian layer which, according to Cowdry (1932), offers more resistance than the keratinized layer. Of these barriers the first is absent in the rumen, the second is thin, while only the third is identical with that of the skin. It is not surprising under these circumstances, especially when it is recalled that the epithelium is continually exposed to semi-fluid ingesta, that absorption takes place apparently more readily than it does through the skin.

SUMMARY

The absorption of sodium ortho-iodo-hippurate has been demonstrated from the rumen of lambs. The epithelium of the rumen is described and compared with that of the skin.

We are greatly indebted to Mr J. F. Fozzard who, by kind permission of Prof. H. A. Harris, performed the radiological part of the work at the School of Anatomy; to Mr A. L. Bacharach and Messrs Glaxo Laboratories who very kindly supplied us with the radio-opaque salt; to Mr J. Freeman who took the microphotographs; and to Mr E. Turner for technical assistance.

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EXPLANATION OF PLATES

Plate 6

Fig. 1. Lamb 2. Exposure taken before opaque drug was given. No shadow is thrown by the bladder.

Fig. 2. Lamb 2. Exposure taken 1½ hr. after the opaque drug was given. A distinct shadow is thrown by the bladder.

Fig. 3. Lamb 2. Exposure taken 3¾ hr. after the opaque drug was given. A dense shadow is thrown by the bladder.

Fig. 4. Lamb 4. Exposure taken 5 hr. after water was introduced into the rumen. The bladder, which contains over 100 c.c. urine, throws only a very faint shadow.

Plate 7

Sections taken from the stomach of a sheep the vessels of which were injected with gelatine carmine.

Fig. 1. Section through a papillae of the rumen (× 100 approx.).

Fig. 2. Section through the glandular mucous membrane of the abomasum (× 100 approx.).

Fig. 3. High power magnification of the epithelium of the rumen (× 450 approx.).

Fig. 4. Section through a single lamina of the omasum (× 100 approx.).

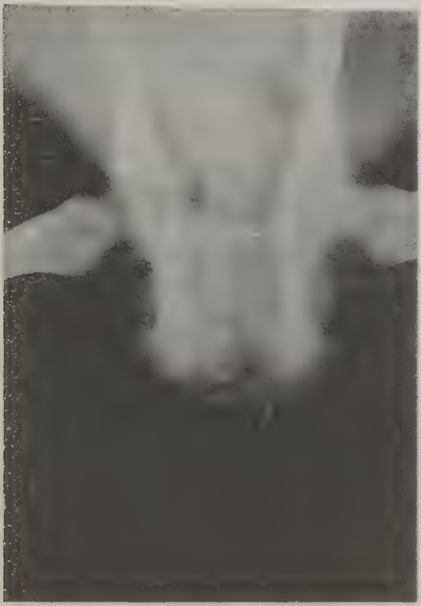

Fig. 1

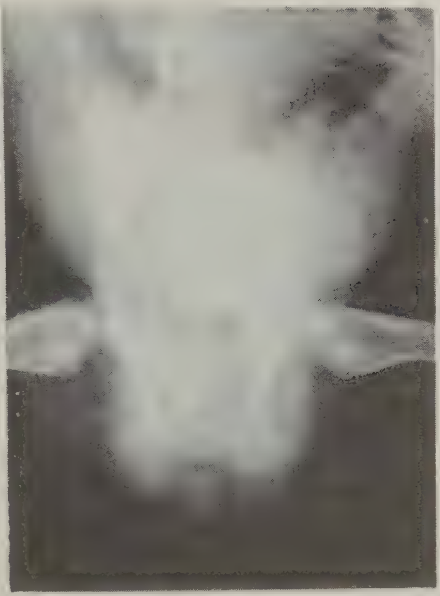

Fig. 2

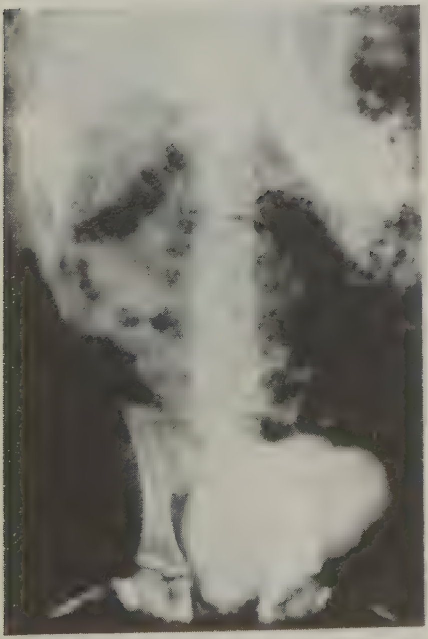

Fig. 3

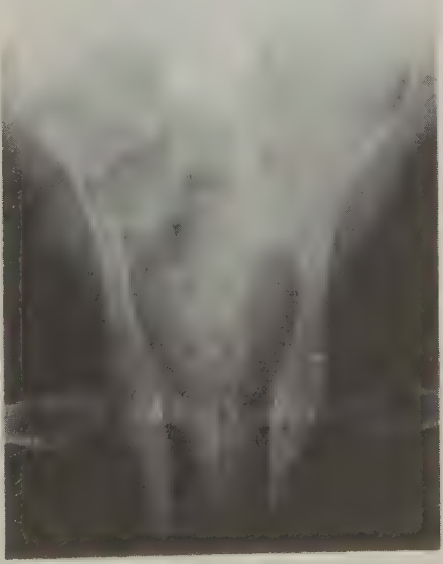

Fig. 4

BARCROFT, McANALLY AND PHILLIPSON—THE ABSORPTION OF SODIUM ORTHO-IODO-HIPPURATE FROM THE RUMEN OF LAMBS (pp. 132–134)

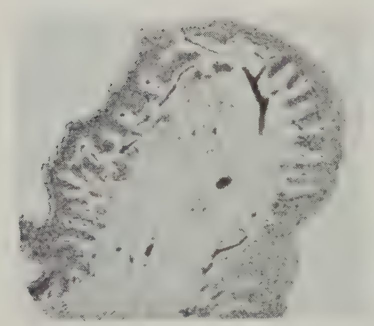

Fig. 1

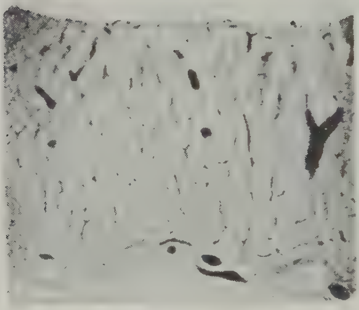

Fig. 2

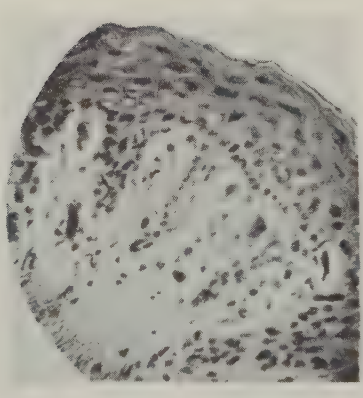

Fig. 3

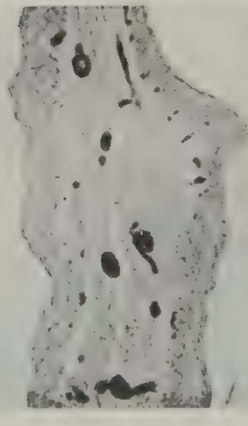

Fig. 4

BARCROFT, McANALLY AND PHILLIPSON—THE ABSORPTION OF SODIUM ORTHO-IODO-HIPPURATE FROM THE RUMEN OF LAMBS (pp. 132–134)

ON INTRASPECIFIC AND INTERSPECIFIC COMPETITION IN LARVAE OF GRAMINIVOROUS INSECTS

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(With Five Text-figures)

I

The behaviour of the beetle, *Rhizopertha dominica*, is adapted to the needs of its larvae in that the sensations which induce the females to oviposit would usually lead to eggs being laid only in environments where sites suitable for larval development are to be found (Crombie, 1942). But the rate of oviposition, even at the extremes of adverse conditions possible in actual populations, is so great that a considerable mortality must occur in the immature stages before the offspring are reduced to numbers which the environment could support (Crombie, 1942). The females of the moth, *Sitotroga cerealella*, appear likewise to have little control over their rate of oviposition (Crombie, 1942). Their ability to select, under natural conditions in the field, an oviposition site suitable for larval development is imperfectly known (King, 1918; Simmons & Ellington, 1927, 1933), but in a confined space it seems adequate (Simmons & Ellington, 1924; Crombie, 1942). The females of neither species avoids ovipositing on already infested grains. In the course of the experiments on oviposition already published (Crombie, 1942) it was frequently noticed that the eggs of either species were deposited next to or even on top of previously oviposited eggs of the same or of the other species (cf. Ullyett, 1936; Salt, 1937; Lloyd, 1939, 1940). Wheat grains already containing larvae of either species also seem to be as acceptable to the females of both species as uninfested grains. Even if the gravid females did avoid ovipositing on previously infested grains her efforts would be vitiated both by the size of the egg batches often placed on one grain, and by the habit of the active first instar larvae of both species of wandering away from the place where they have hatched. The rudimentary nature of 'parental care' in these two species of insect means that the larvae must be adapted, particularly during the early instars, to an independent existence. This will expose them, in choosing and occupying situations in which to complete their development, to the competition of other larvae similarly engaged. In this paper the term *superinfestation* will designate the cases in which one wheat grain is inhabited simultaneously by two or more young of the same species of insect. The term *multiple infestation* will designate the cases in which one wheat grain is inhabited by the young of two or more different species of phytophagous insect (cf. Smith, 1929). It is important to know firstly, whether the larvae themselves avoid multiple or superinfestation, and if so, by which senses they perceive the larva already in occupation of the grain; and secondly, if multiple and superinfestation are not avoided, how overcrowding affects their development and what is the outcome of competition between the two species in mixed populations (cf. MacLagan, 1932; Timofeeff-Ressovsky, 1933, 1935; L'Héritier, 1937; Bodenheimer, 1938). Overcrowding may evidently affect living organisms through the limitation of food, oxygen or space (leading to reflex stimulation caused by the perception of other individuals, e.g. by actual contact), or the accumulation of excretory products ('conditioning' of the medium) (Allee, 1931, 1934; Hammond, 1938, 1939; Clements & Shelford, 1939; Park, 1939, 1941; MacLagan, 1941). When insect larvae are exposed to such conditions this may lead to the death of individuals before reaching maturity (MacLagan & Dunn, 1935; Salt, 1936); the reduction in size of the pupae and resulting adults (Weidling, 1928; Holdaway, 1930; Salt, 1932*a*), or of the vigour and longevity of the latter; the lowering of the fecundity of the adult females (Hofmann, 1933; Dunstan, 1935); retardation (Landowski, 1938) or stimulation (Michal, 1931), of the rate of development; changes in the sex ratio of the emerging adult population (Herms, 1928; Brandt, 1937; Flanders, 1939; Graham, 1939); or failure to make full use of the food reserves of the medium because of competition for space. The latter may lead to survival being lower at high than at low densities.

The experimental conditions in these experiments were the same as those described in a previous paper, to which reference should be made (Crombie, 1942). All the experiments were performed in a dark incubator at 30° C. and 70% R.H.

The larvae of both species usually bore into a suitable object (e.g. a wheat grain) in the first instar, and generally remain there, feeding upon the interior of the grain, until they have completed their development (Barnes & Grove, 1916; Fletcher, 1920; Back, 1922). There are usually four larval instars in *Rhizopertha* followed by a prepupal stage which does not involve moulting. This moults to form the pupa, which is followed by the adult. The larval instars are with practice easily distinguished by eye by means of the characteristics described by Potter (1935), but were checked by measuring the head capsule. Under our experimental conditions the average duration of the instars was as follows: egg, 6 days; first larval instar, 6 days; second, 6 days; third, 5 days; fourth, 4 days; prepupa, 1 day; pupa, 7 days; total, 35 days. In *Sitotroga* there are also usually four larval instars, which are with practice readily distinguishable by eye. As before, they were when necessary checked by measuring the head capsule. The average duration of the instars under our experimental conditions was as follows: egg, 3 days; first larval instar, 6 days; second, 6 days; third, 5 days; fourth, 7 days; pupa, 5 days; total, 32 days (Crombie, 1943). The period from hatching to emergence was thus 29 days for each species. This period was sometimes abnormally extended (vide infra). In both species the pupa is found in the cavity made by the feeding larva. Escape from the grain by the adults of *Sitotroga* is accomplished by the act of emerging from the pupa. The adults of *Rhizopertha* usually remain inside the grains for a few days while their cuticle hardens before eating their way out. The larvae of *Rhizopertha* can enter intact wheat grains only in the first instar. This can be accomplished by the larvae of *Sitotroga* in all instars, the resulting mortality decreasing with age (Back, 1922). The mortality among first instar larvae of both species as a result of such an attempt may be as high as 50%.

The results of all statistical tests in this paper are given in terms of the value of p, which represents the probability of obtaining as great a deviation by random sampling as that observed in the experiment. A probability (p) of 5% is usually taken as the arbitrary division between significance and non-significance, so that when $p < 0.05$ the difference between the values compared may be regarded as significant (Simpson & Roe, 1939).

II

Choice of food by larvae. Single larvae were confined in cells made by gumming a cover-slip over a glass ring. A series of such cells was stood on a ground-glass plate. Each cell contained besides the larva two objects, each composed of different substances, between which the larva had to make a choice of which it was to enter. After 6 hr. in the incubator the cells were examined and the positions of the larvae noted. The latter always bored into one or other of the objects. Twenty *Rhizopertha* larvae of each instar were offered the choice of each of the following pairs of substances: a piece of wheat grain and a piece of cork cut to the same shape and size; a false grain made of plaster of Paris mixed with wheat flour and a false grain made of plain plaster of Paris (Crombie, 1941). In both experiments all twenty larvae of each instar chose the wheat. The distinction made between the false grains of plaster containing wheat flour and those composed of plaster alone suggests the existence of a chemoreceptive sense, since the only difference between them is provided by the food. The first experiment was repeated with twenty *Sitotroga* larvae of each of the four instars, and in each case the wheat was preferred to the cork twenty out of twenty times. Twenty first instar larvae of each species, respectively, were now given the choice of a piece of bean seed and a piece of cork of the same shape and size, and of a piece of wheat grain and a piece of bean seed of the same shape and size. All the *Rhizopertha* and eighteen of the *Sitotroga* larvae chose the bean to the cork; seventeen of the *Rhizopertha* and all of the *Sitotroga* chose the wheat to the bean. The larvae of all instars of both species can thus recognize objects containing food, and the first instar larvae of both species are able to distinguish between different kinds of food.

III

As already stated, the proportion of first instar larvae of either species which manage to enter intact wheat grains may be as low as 50%. With such a high mortality the experiments to be described in this paper could not have been performed. It was necessary therefore to damage the test in some standardized and repeatable way, so that the larvae could enter more easily. This was done by gently tapping the grains until the test was just broken by a crack. The larvae almost invariably entered the grains in the damaged regions and mortality during entry was reduced almost to zero. The grains were treated in this way in all the experiments described in this paper. This may appear to introduce an unnatural condition, but we are chiefly interested in the competition inside the wheat grains, and not in mortality which is the result of the accident of their possessing a hard test. This study must be regarded, however, as a study in competition for 'cracked grains', and not for intact wheat.

Avoidance of super- or multiple infestation. 150 wheat grains were placed into each of three dishes so that they were one layer deep. Into one of these dishes (*a*) were introduced 100 first instar *Rhizopertha* larvae, into a second dish (*b*) 100 first instar *Sitotroga* larvae, and into the third (*c*) 50 first instar larvae of each species. After 24 hr. in the incubator the grains were dissected and the positions of the larvae noted. The results are given in Table 1. The number of grains containing 0, 1, 2, 3, etc., larvae, assuming that the latter distribute themselves at random among the grains, were calculated from the Stoy formula where x is the number of larvae and N the number of grains (Appendix to Salt, 1932*b*). Each of the observed values (*a*), (*b*) and ((*c*): total) were compared with these calculated values by means of χ^2, and none of them was significantly different from the latter ($p > 0.05$). In (*c*) the members of each species entered the grains in practically equal numbers. It may be concluded therefore that the larvae of neither species avoid either superinfestation or multiple infestation (cf. King, 1918; Simmons & Ellington, 1925; Bodenheimer, 1930). Each species, however, possesses a kind of behaviour by which overcrowding may be prevented, viz. migration. The habit of migration of *Sitotroga* larvae from the grains they were inhabiting has been remarked upon by Back (1922), but no reference has been found to it in *Rhizopertha*.

Causes of migration of Rhizopertha *larvae*. The non-avoidance of superinfestation by *Rhizopertha* larvae was confirmed by the following experiment: each of a number of first instar larvae was given the choice between a fresh grain and a grain (marked with a spot of ink) which contained three living larvae. The two grains were placed side by side on a ground-glass plate, and covered by one of the cells described above. The larvae cannot escape from such cells, a series of which were arranged on each glass plate. After 6 hr. the grains were dissected by slicing with a sharp scalpel under a binocular microscope, and the positions of the larvae noted. In approximately half the cells (23 out of 50) the fresh grains contained one larva while in the other half (27 out of 50) the infested grains contained four larvae, showing that half the larvae had entered each set of grains. There was therefore no avoidance of the infested grains. The point to be decided now was whether or not it is the presence of other larvae in the same grain which causes a larva to migrate. Fifty first instar larvae were placed separately under cells with one wheat grain each, and the whole put into the incubator for 6 hr. The cells were then examined and all the larvae were found to have entered the grains. A fresh grain (marked) was now introduced into each cell and the whole returned to the incubator once more. Every 2 days until adults emerged the fresh grains were removed, dissected and replaced by other fresh grains. No larvae were found in the fresh grains and eventually forty-two adults emerged from the infested grains. Those from which adults had not emerged were then dissected: dead first instar larvae were found in four of them, dead larvae of later instars in two more, while the other two contained dead, somewhat deformed adults. It may be concluded then that migration occurs only when two or more larvae enter the same grain. Mortality may occur, however, in larvae occupying grains singly.

Table 1. *Distribution of larvae among wheat grains*

Larvae	No. grains containing *x* larvae			
	0	1	2	3
Calculated	77	52	17	4
(*a*) *Rhizopertha*	74	59	14	3
(*b*) *Sitotroga*	71	63	12	4
(*c*) *Rhizopertha*	—	29	6	2
with *Sitotroga*	—	32	6	1
Total	74	61	12	3

If larvae migrate thus owing to the influence of other larvae occupying the same grain, it may be asked by what sense a larva perceives that there is another larva present. There are three possibilities: chemoreception, 'hearing' or stimulation from mechanical vibrations (cf. Minnich, 1925) and stimulation resulting from actual contact. Now if larvae migrate after detecting the presence of other larvae by chemoreception, freshly killed larvae ought perhaps to cause migration in the same way as living larvae. A number of freshly killed larvae were introduced into a hole made in a wheat grain, a living first instar larva placed beside each such grain, and both covered with a cell. After 6 hr., during which the larvae entered the grains, a fresh grain (marked) was introduced into each cell. After 48 hr. the grains (50 in all) were dissected and all the larvae were found still in the grains containing the dead larvae. Chemoreceptive or other stimuli from the latter do not therefore cause migration.

The effect of mechanical vibrations was investigated as follows. Two grains, each containing three first instar larvae, were firmly cemented together. Fifty such pairs were set up. Each of these, together with five fresh grains to each, was then placed on a glass plate and covered with a cell. After 2 days the grains were dissected and the positions of the larvae noted. Now as described below, the number of larvae which migrate from or die in a grain increases with the initial number present (Table 2). Mechanical vibrations are not likely to cause death, so we may omit the dead larvae for the moment. If such vibrations were the cause of migration one might expect a similar increase in migration with an increase in this factor. Now one would expect that the amount of vibration or noise produced by six larvae in two grains cemented together, would be greater than that produced by three larvae in a single grain. However, the average number of larvae migrating per grain from the cemented grains was 0·32, which is not significantly different from the number, 0·31 per grain, of larvae migrating from single grains each containing initially three larvae. There was also no significant difference between the total number of larvae killed and migrating per grain in the two experiments, this value being 0·4 and 0·38 for cemented pairs and single grains respectively (Table 2). Mechanical vibrations do not therefore seem to be the cause of migration.

The probable importance of actual contacts between larvae as a cause of migration becomes immediately apparent. The reaction of one larva to the presence of another was observed as follows. When two larvae in the first, second, third or fourth instars were put together into a small hole drilled in a wheat grain and watched under a binocular microscope, they were often seen to attack each other with their mandibles, and eventually either one or both left the hole. When a larva entered such a hole it always went to the bottom and turned round so as to face outwards. Other larvae trying to enter the hole were fiercely attacked. Sometimes such combats resulted in the body wall of one of the antagonists becoming punctured and its bleeding to death. In their tunnels in wheat grains larvae of all instars were always found curled up with the head facing towards the way they had entered. Furthermore, in all grains dissected during the experiments to be described, whenever two larvae were found in the same tunnel at least one of them was always dead. It thus seems probable that whenever two larvae meet within a grain they will attack each other, with the result that either or both will migrate or be killed.

Table 2. *Effect of density on the reduction in numbers of* Rhizopertha *larvae competing for the same wheat grain*

(1) Initial no. larvae per grain (i)	(2) No. grains	(3) Total no. larvae	(4) Total no. larvae killed and migrating	(5) Observed average no. larvae killed and migrating per grain (p_i)	(6) Observed average % larvae killed and migrating per grain (p_i/i)	(7) Calculated average % larvae killed and migrating per grain (p_i/i)	(8) % larvae in column 4 which migrated
Instar I 1	100	100	0	0	—	—	—
2	106	212	17	0·16	8	8	89
3	42	126	16	0·38	12·7	16	81
4	38	152	35	0·92	23	24	66
5	13	65	33	1·69	34	32	55
8	10	80	46	4·6	57·5	56	31
Instar II 2	52	104	12	0·23	11·5	11·5	83
3	14	42	9	0·64	16·3	23	78
4	13	52	18	1·38	34·5	34·5	50
Instar III 2	41	82	18	0·44	22	22	78
3	26	78	28	1·08	36	44	68
Instar IV 2	24	48	14	0·58	29	29	86
3	13	39	18	1·38	46	58	72

The effect of density upon the migration and death of Rhizopertha *larvae.* Different numbers of larva were introduced into wheat grains as follows: the requisite number of larvae was placed with a wheat grain on a ground-glass plate and covered with a cell. A number of such cells were set up. After 6 hr. in the incubator the grains were examined. All the larvae had usually entered them by this time, grains in which they had not done so being rejected. Five fresh grains (each marked with a spot of ink) were now introduced into each cell and the whole returned to the incubator for 48 hr. At the end of this period the grains were all dissected by slicing with a sharp scalpel (the original infested grains being examined first in order to minimize errors due to the extension of the 48 hr. period by the time taken in dissection) and the positions of the larvae noted. This provided data about the number of larvae killed in and migrating from each original grain during a constant period of time. This procedure was followed with larvae in the first, second, third and fourth instars. The first instar larvae had recently hatched, the others having recently moulted. The results are given in Table 2. With initially only one larva per grain no migration or death occurred during this 2-day period. During this period of their lives both these effects were therefore apparently due to the presence of more than one larva in each grain, and the increase in migration and death with increased initial numbers was due to crowding.

Now if larvae do not perceive other larvae (e.g. by chemoreception or 'hearing') before actual contact occurs, or, more precisely, if they do not react to perception at a distance, if it occurs, by dying or migrating, then contacts between larvae in the same grain would occur only as a result of random encounters. The relationship between the initial number of larvae present and the number killed and migrating would then depend on the relative probability of their encountering each other when there were different initial numbers of larvae per grain. The determination of the relative probability depends simply on a consideration of the number of encounters that could take place between any two individual larvae in a grain containing i larvae. This number of encounters is equal to $i\,(i-1)$, and if the number of larvae killed and migrating per grain is p_i, then

$$p_i = ki\,(i-1), \quad p_i/i = k\,(i-1), \tag{1}$$

which means that the proportion of larvae killed and migrating is linearly related to the initial number of larvae per grain. The value of k may be calculated at any value of i from the observed value of p_i, and this value of k may then be used to calculate the values of p_i at other values of i. The reasonableness of the above hypothesis will then be measured by the agreement between the observed and calculated values of p_i or p_i/i. The values of k calculated from the data for first, second, third and fourth instar larvae in Table 2 when $i=2$ are, respectively, 0·08, 0·115, 0·22 and 0·29. The values of p_i/i (expressed as percentages) calculated, with different values of i, from these values of k respectively, appear in column 7 of Table 2, while the observed percentages of larvae killed and migrating per grain are shown in column 6. An inspection of Fig. 1, which sets out the relation between i and p_i/i, shows that the agreement of observed and calculated values is very close for first instar larvae, but less so for the older larvae. The goodness of fit between the observed and calculated value of p_i (which are not shown here) was tested by calculating the values of χ^2, and there proved to be no significant difference between the two sets of values for either the first or the second instar. This test could not be applied to third and fourth instar larvae. These results show that at any rate with first and second instar larvae the hypothesis that death and migration occur as a result of random encounters within the grain is a possible one, and bearing in mind the absence of any other probable explanation of the experimental data it is reasonable to adopt it as correct.

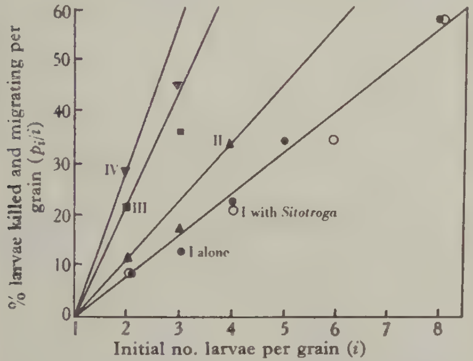

Fig. 1. The relationship between density and the reduction in numbers of *Rhizopertha* larvae developing in wheat grains: instars I, II, III and IV alone (Table 2, column 6), and instar I competing with *Sitotroga* (Table 4, column 7). The lines are drawn through the theoretical points (Table 2, column 7).

Two further points of interest in Table 2 may be noted. First, as larval density increases the proportion of larvae killed as a result of encounters increases, while the proportion migrating decreases (column 8). Secondly, the number of larvae killed and migrating per grain increases with later instars (column 5). This may be because the larger size of later instars increases the probability of encounter. Finally, it may be mentioned that the number of larvae killed and migrating from grains which had been cut either in half or in quarters was significantly greater than from the whole grains. These observations are of interest merely in that they confirm the foregoing results, so that no details will be given.

The effect of density upon the migration and death of Sitotroga *larvae.* The existence of larval fighting in this species was observed nearly 200 years ago by Duhamel & Tillet (1762). These authors remark that they often found three or four dead larvae in grains of which only one living larva had taken possession. On one occasion two larvae were seen apparently fighting. After several minutes one was dead and the other was seen working its way into the grain. They were never able to find more than one living larva per grain. Similar observations have been made by Flanders (1933). Fighting, sometimes resulting in death, has been observed here, also, among larvae confined together in a small hole bored in a wheat grain. In all the grains dissected during the experiments described below two living larvae were never found in the same tunnel, although a living and a dead larva, or two dead ones, were sometimes found together. The experiments described above with *Rhizopertha* were now repeated with this species, but only with first instar larvae. As the results were similar to those obtained with the former species, and negative, no details will be given. The larvae did not avoid entering grains already containing other larvae, and chemoreceptive and 'auditory' stimuli were, as before, apparently not causes of migration. An experiment to determine whether migration or death occurred in grains containing only a single larva was carried out as with *Rhizopertha*. No migration occurred, and from the fifty grains forty-three adults eventually emerged (cf. Barnes & Grove, 1916; Fletcher, 1920). On the dissection of those from which adults had not emerged four dead first instar larvae, one dead third or fourth instar larva, one dead pupa, and one dead, deformed adult were found.

The average reduction in numbers of larvae per grain by migration or death during a fixed period of time under different degrees of crowding was now determined as before. The results are shown in Table 3. Seven out of a hundred larvae died, during the 2-day experimental period, in the grains containing initially only one larva. This means that 7% of the larvae died, without encounters with other larvae, in the first 2 days after hatching. There seems to be no reason why such mortality should not occur at any density, so that we must subtract from the total number of larvae killed and migrating at any density (column 4), 7% of the initial number of larvae introduced into the grains. The remainder is the number of larvae killed and migrating as a result of random encounters (column 5). The value of k was calculated as before from the observed value of p_i when $i=2$, and in this case $k=0{\cdot}09$. The values of p_i/i (expressed as percentages) calculated from this value of k at different values of i are given in column 8, while the observed percentages of larvae killed and migrating per grain appear in column 7. An inspection of Fig. 2 shows, as before, the close agreement between observed and calculated percentages. The goodness of fit between observed and calculated values of p_i (not shown here) was tested by calculating the value of χ^2, and there proved to be no significant difference between them. It is therefore reasonable to believe that the elimination of *Sitotroga* first instar larvae also takes place as a result of random encounters within the grain. As before, the proportion of larvae killed as a result of encounters increases with increasing density, while the proportion migrating decreases (column 9). The rate of migration and death of *Sitotroga* is significantly higher than that of *Rhizopertha* (χ^2 corresponds to $p<0{\cdot}01$).

Table 3. *Effect of density on the reduction in numbers of first instar* Sitotroga *larvae competing for the same wheat grain*

(1) Initial no. larvae per grain (i)	(2) No. grains	(3) Total no. larvae	(4) Observed total no. larvae killed and migrating	(5) Total no. larvae killed and migrating after chance encounters	(6) Observed average no. killed and migrating per grain after chance encounters (p_i)	(7) Observed average % larvae killed and migrating per grain after chance encounters (p_i/i)	(8) Calculated average % larvae killed and migrating per grain after chance encounters (p_i/i)	(9) % larvae in column 5 which migrated
1	100	100	7	0	—	—	—	—
2	69	138	22	12·4	0·18	9	9	81
3	40	120	30	21·6	0·54	18	18	70
4	20	80	25	19·4	0·97	24·3	27	57
6	16	96	54	41·3	2·58	43	45	42
8	19	152	116	105·7	5·54	69·3	63	19

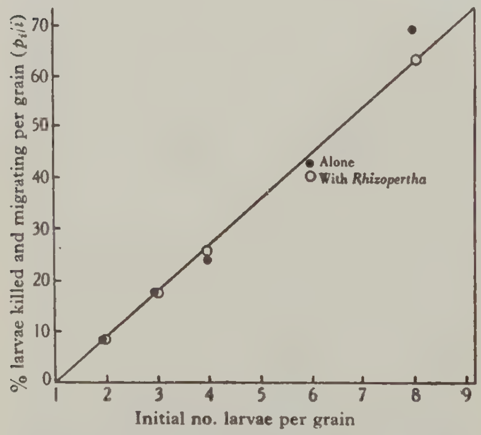

Fig. 2. The relationship between density and the reduction in numbers after chance encounters of first instar *Sitotroga* larvae developing in wheat grains alone (Table 3, column 7) and in competition with *Rhizopertha* (Table 4, column 7). The line is drawn through the theoretical points (Table 3, column 8).

The effect of competition between Rhizopertha *and* Sitotroga *larvae upon the migration and death of both species.* The above experiment was now repeated except that first instar larvae of both *Rhizopertha* and *Sitotroga* were introduced into the grains together in equal numbers as shown in Table 4. The values for *Sitotroga* are consistently higher than those for *Rhizopertha*, although the difference between them is not statistically significant. The expected values of p_i/i were calculated from the values of k obtained from Tables 2 and 3, respectively, and are shown in column 8 of Table 4. An inspection of Figs. 1 and 2 shows that, as before, there is a close agreement between observed and calculated percentages. The calculation of χ^2 showed also that there is no significant difference between observed and calculated values of p_i for either species (not shown here). The values of p_i/i are (for both species) closely similar whether they are competing intraspecifically or interspecifically, which shows that the degree to which the larvae of either species are affected depends upon that species rather than upon the competing species. The *Rhizopertha* appear to be slightly the more successful in competition. It should be mentioned that the larvae of either species will attack those of the other. In all the grains dissected in the experiments described below, whenever two larvae were found in the same tunnel at least one of them was always dead.

Observations on migration and death throughout development. By the usual method four freshly hatched *Rhizopertha* larvae were introduced into each of a number of wheat grains. Five fresh grains (marked) were then placed in each of the cells containing one infested grain, and the whole returned to the incubator. The fresh grains were dissected, being replaced by an equal number of new fresh grains, once every 4 days, until adults had ceased emerging from the infested grains. The number of larvae migrating to the fresh grains over the whole developmental period was thus observed, instars being checked by measuring the width of the head capsule. Most of the adults had emerged by the 40th day but a few emerged later. The infested grains were all dissected on the last day of observation. The results are shown in Table 5. Most of the migration took place in the first and second instars, and migration had practically ceased by the 20th day. By this time most of the larvae would be entering the prepupal stage. Of the original four larvae per grain the average number which survived to become adults was only 1·14 or 28·5 %, 26·1 % of the larvae migrating and 45·4 % being killed or dying. Some of the latter, in various instars including one dead, deformed adult, were found on dissecting the infested grains at the end of the experiment.

Table 4. *Effect of density on the reduction in numbers of larvae of* Rhizopertha *and* Sitotroga *competing for the same wheat grains*

(1) Initial no. 1st instar larvae per grain (i)	(2) No. grains	(3) Total no. larvae	(4) Observed total no. larvae killed and migrating	(5) Total no. larvae killed and migrating after chance encounters	(6) Observed average no. larvae killed and migrating per grain after chance encounters (p_i)	(7) Observed average % larvae killed and migrating per grain after chance encounters (p_i/i)	(8) Calculated average % larvae killed and migrating per grain after chance encounters (p_i/i)	(9) % larvae in column 5 which migrated
Rhiz. 1	—	72	6	6	0·08	8·3	8	83
Sit. 1	—	72	12	7	0·10	9·7	9	86
Total 2	72	144	18	13	0·18	—	—	85
Rhiz. 2	—	96	19	19	0·39	19·8	24	79
Sit. 2	—	96	31	24·3	0·51	25·4	27	78
Total 4	48	192	50	43·3	0·90	—	—	78·5
Rhiz. 3	—	69	23	23	1·0	33·4	40	48
Sit. 3	—	69	33	27·5	1·2	40·0	45	55
Total 6	23	138	56	50·5	2·2	—	—	51
Rhiz. 4	—	40	23	23	2·3	57·5	56	39
Sit. 4	—	40	28	25·2	2·52	63·0	63	44
Total 8	10	80	51	48·2	4·82	—	—	42

Table 5. *Observations on the migration of* Rhizopertha *from wheat grains throughout development (initially four 1st instar larvae per grain; 43 grains)*

Instar	No. larvae migrating after x days						Total	Average per grain
	4	8	12	16	20	32		
I	21	4	—	—	1	—	26	0·6
II	—	4	3	2	2	—	11	0·26
III	—	—	1	1	—	1	3	0·07
IV	—	—	—	4	—	1	5	0·12
Total	21	8	4	7	3	2	45 = 26·1 %	1·05
Total no. adults emerging							49 = 28·5 %	1·14
Total no. larvae migrating + adults emerging							94	2·19
Total no. larvae introduced into the 43 grains							172	4·0
∴ no. immature insects killed							78 = 45·4 %	1·81

This experiment was now repeated with *Sitotroga*. The results (Table 6) here are similar to those just described. Instars were checked as before by measuring the width of the head capsule. Most of the adults had emerged by day 36, but some appeared after longer periods. Migration took place chiefly in the first and second instars and ceased by day 20. The larvae of this species would be in the fourth instar and perhaps beginning to pupate by this time. Of the original four larvae per grain an average number of 1·27 per grain or 31·6 % survived to emerge as adults, 22·5 % of the larvae migrating and 45·9 % dying or being killed. Some of the latter, in various instars including one dead deformed adult and one dead pupa, were found on dissecting the infested grains at the end of the experiment.

Table 6. *Observations on the migration of* Sitotroga *from wheat grains throughout development (initially four 1st instar larvae per grain; 30 grains)*

Instar	No. larvae migrating after *x* days					Total	Average per grain
	4	8	12	16	20		
I	14	1	1	1	—	17	0·57
II	—	—	3	3	—	6	0·2
III	—	—	—	2	1	3	0·1
IV	—	—	—	—	1	1	0·03
Total	14	1	4	6	2	27 = 22·5 %	0·9
Total no. adults emerging						38 = 31·6 %	1·27
Total no. larvae migrating + adults emerging						65	2·17
Total no. larvae introduced into the 30 grains						120	4·0
∴ no. immature insects killed						55 = 45·9 %	1·83

Table 7. *Observations on the migration from wheat grains of* Rhizopertha *and* Sitotroga *(in competition) throughout development (30 grains)*

Instar	No. larvae migrating after *x* days										Total			Average per grain		
	4		8		12		16		20							
	R.	*S.*	*R.*	*S.*	*R.*	*S.*	*R.*	*S.*	*R.*	*S.*	*R.*	*S.*	Total	*R.*	*S.*	Total
I	7	8	1	2	—	—	1	1	—	—	9	11	20	0·3	0·37	0·67
II	—	—	1	1	1	3	—	—	—	—	2	4	6	0·07	0·13	0·2
III	—	—	—	—	—	—	2	—	1	1	3	1	4	0·1	0·03	0·13
IV	—	—	—	—	—	—	—	—	—	1	—	1	1	—	0·03	0·03
Total	7	8	2	3	1	3	3	1	1	2	14	17	31	0·47	0·56	1·03
% migrating											23·3	28·4	25·8	—	—	—
Total no. adults emerging											18	19	37	0·6	0·63	1·23
% adults emerging											30	31·6	30·8	—	—	—
Total no. larvae migrating + adults emerging											32	36	68	1·07	1·2	2·27
Total no. larvae introduced into the 30 grains											60	60	120	2	2	4
∴ no. immature insects killed											28	24	52	0·93	0·8	1·73
% immature insects killed											46·7	40	43·3	—	—	—

A third experiment was now carried out which was a repetition of the preceding two except that of the initial four freshly hatched larvae per grain two were *Rhizopertha* and two *Sitotroga*. The results are given in Table 7. As before migration occurred chiefly in the first and second instars and had ceased by day 20. Of the original four larvae per grain the average number which survived to become adults was 1·23 per grain or 30·8 %. Of these survivors 48·7 % were *Rhizopertha* and 51·3 % *Sitotroga*. The proportion of the original larvae migrating was 25·8 % of which 45 % were *Rhizopertha* and 55 % *Sitotroga*; while the proportion dying or being killed was 43·3 % of the original number, of which 54 % were *Rhizopertha* and 46 % *Sitotroga*. As before some of the latter, in various instars including one dead pupa of each species, were found on dissecting the infested grains at the end of the experiment.

What has been observed here is what would happen in a culture in which a large number of wheat grains were present, such as in experimental populations growing in wheat media (unpublished) or in stores of wheat in which these insects are pests. The experiments have provided data about the amount of reduction in numbers for which each of the two methods, migration and death, is responsible, the instars during which migration occurs, the number of larvae capable of successfully completing their development in one grain, and the outcome of competition between *Rhizopertha* and *Sitotroga.*

IV

The effect of larval density upon the survival of Rhizopertha *and* Sitotroga. Increasing numbers of freshly hatched larvae were introduced into different wheat grains and each wheat grain placed in a small vial. The whole was then placed in the incubator. There were no fresh grains, each of the vials containing one infested grain only. The vials were examined at regular intervals and the numbers and dates of emergence of adults recorded. The adults were sexed and weighed. We thus have records of the total number of adults emerging, the number emerging per grain, the developmental period, sex ratio and weight. In Table 8 are given the results of experiments on the effect of larval density upon the survival of (*a*) *Rhizopertha* and (*b*) *Sitotroga* separately. In both cases when there was initially one larva per grain only 80% of the larvae, or 0·8 per grain, survived to become adults. Now in the experiments carried out to investigate whether migration would occur with initially only one larva per grain (vide supra), with both species four of the corpses found in the grains from which no adults emerged were first instar larvae. If these had survived 46 out of 50 or 92% of the *Rhizopertha* larvae, and 47 out of 50 or 94% of the *Sitotroga* larvae would have become adults. When the larvae were introduced into the grains in the second instar, with initially one larva per grain 94% of the *Rhizopertha* and 96% of the *Sitotroga* larvae survived to become adults (Table 8). Therefore there is a certain mortality in the first instar which is independent of larval encounters.

Table 8. *The effect of density upon the survival of the larvae of* Rhizopertha *and* Sitotroga *developing in wheat grains*

(1) Initial no. larvae per grain (i)		(2) No. grains	(3) Total no. larvae	(4) Total no. adults emerging	(5) Average no. adults emerging per grain	(6) % larvae developing into adults $(1-P)\times 100$	(7) % survivors with development retarded by over 21 days
			(*a*) *Rhizopertha* alone				
Instar I	1	178	178	143	0·8	80	1·5
	2	72	144	83	1·15	57·7	2·4
	3	74	222	92	1·24	41·5	1·08
	4	75	300	87	1·16	29	1·15
	6	40	240	49	1·22	20·5	4·1
	8	40	320	41	1·03	12·8	4·9
	10	40	400	50	1·25	12·5	0
	14	30	420	32	1·07	7·6	3·1
	20	36	720	40	1·11	5·6	2·5
Total (2–20)		407	2766	474	1·16	17·2	1·5
Instar II	1	100	100	94	0·94	94	2·12
	2	40	80	43	1·08	54	0
	3	29	87	39	1·34	45	0
	4	18	72	26	1·44	36	3·85
Total (2–4)		87	239	108	1·24	135	2·8
			(*b*) *Sitotroga* alone				
Instar I	1	130	130	104	0·8	80	3·9
	2	80	160	88	1·1	52	14·8
	3	66	198	67	1·01	34	7·5
	4	63	252	69	1·1	27·5	11·6
	6	49	294	60	1·22	20·5	15·0
	8	44	352	49	1·11	14	16·3
	10	40	400	52	1·3	13	21·0
	14	30	420	29	0·97	6·9	0
	20	30	600	32	1·07	5·6	6·25
Total (2–20)		402	2676	446	1·1	16	12·1
Instar II	1	100	100	96	0·96	96	3·1

Now let i be the initial number of first instar larvae per grain, P be the proportion dying, and a be the average number of insects surviving per grain when $i > 1$. Then if the larvae are eliminated at random (i.e. the probability of survival of any insect is inversely proportional to the initial number present) but a remains constant, the proportion of larvae surviving will be

$$(1 - P) = \frac{a}{i}. \qquad (2)$$

When the value of a has been determined the expected values of $(1 - P)$ for any value of i above 1 may be calculated. The agreement of expected and observed values of $(1 - P)$ will depend on the constancy of a. The value of a for *Rhizopertha* was 1·16 and for *Sitotroga* 1·1. It is obvious from column 5 of Table 8 that for both species there is no regular change in a with density (cf. Jacobi, 1939). The observed values of $100\ (1 - P)$ shown in column 6 were, however, compared with the expected values for each species (not shown) by means of χ^2, and there proved to be no significant difference between them for either species. There was also no significant difference between the observed values of $(1 - P)$ for the two species, so that the two observed values of a may be considered as equal. The observed relationship between i and $100\ (1 - P)$ for each species is shown in Fig. 3. The value of $(1 - P)$ approaches but never reaches zero.

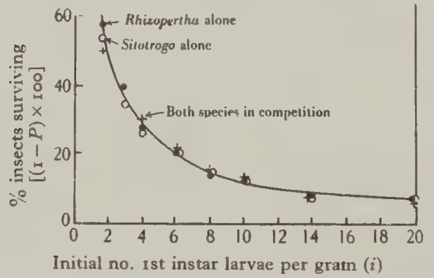

Fig. 3. The relationship between density and the survival of first instar larvae of *Rhizopertha* and *Sitotroga* developing in wheat grains alone (Table 8, column 6) and in competition with each other (Table 9, column 7). The line is drawn through the theoretical values of $100\ (1 - P)$ when $a = 1{\cdot}2$ (equation (2)).

The majority of the adults of both species emerged within a few days of each other. In *Rhizopertha* over 95 % emerged between 28 and 38 days after hatching, the mean developmental period of the insects emerging within these limits being 32 days. Over 86 % of the *Sitotroga* emerged between 26 and 36 days after hatching, the mean developmental period of the insects emerging between these limits being 29 days. The term 'developmental period' refers here to the time elapsing between the hatching of the egg and the emergence of the adult from the pupa. This can be accurately determined for *Sitotroga* since in the act of emerging from the pupa the adult also escapes from the wheat grain in which the pupa is lying. But owing to the habit of remaining inside the grain for a few days after emergence from the pupa while the adult cuticle hardens, the precise moment of emergence from the pupa cannot be accurately determined for *Rhizopertha* which have pupated inside wheat grains. What is observed is the moment when the adult eats its way out of the grain, and consequently the values for developmental period are too high. From comparison with data obtained with insects developing in flour, where the precise moment of emergence from the pupa can be observed, the period between emergence and escaping from the grains is approximately 3 days, which makes the actual mean developmental period of *Rhizopertha* approximately 29 days, or equal to that of *Sitotroga*. The insects not emerging within the limits of the majority of each species as defined above, had widely divergent developmental periods, always longer than those of the majority. The longest developmental period observed for *Rhizopertha* was 90 days, and for *Sitotroga* 120 days. As the developmental periods of these minorities were widely scattered, they were disregarded in calculating the mean developmental periods at each density. The latter did not vary significantly at any density from the mean values given. Now in column 7 *b* are given the percentages of the surviving *Sitotroga* whose developmental periods were more than 21 days longer than the average. That is, these insects emerged after the 50th day from hatching. The correlation coefficient between the proportion of insects with such retarded development (column 7 *b*) and the average number of adults emerging per grain (column 5 *b*) was calculated, and proved to be significantly different from zero at the 1 % level, and positive. The two sets of variables are therefore highly positively correlated. On the other hand, with *Rhizopertha* the number of survivors emerging more than 21 days after the average (i.e. after day 53) was never greater than two at any density, and no calculations can be based on such small numbers. The reason for choosing the period of 21 days will appear later. With *Sitotroga*, in the majority of the grains from which more than one adult emerged, one of the insects had an average rate of development while the rate of development of the other was retarded. The result was that the two insects which developed successfully had widely different rates of development. In this experiment there were actually 56 grains from which more than one *Sitotroga* adult emerged. The dates of emergence of the adults which had developed in one grain were separated by more than 21 days in 27 = 48·2 % of these grains, by 14–21 days in 16 = 28·6 %, and by less than 14 days in 13 = 23·2 %. In the grains in which only one insect completed its development the adults emerged within 14 days of each other in 390 = 90 % out of 434 grains. In *Sitotroga*, therefore, if two larvae which have entered the same grain have widely different developmental periods it will be more probable that both of them will successfully complete their development, than it will be if their developmental periods are nearly equal. This is possibly because larvae of approximately the same instar are less tolerant of each other than are those of widely different instars (cf. Flanders, 1933). One larva with a retarded rate of development in a grain containing several normal larvae would therefore have a greater chance of survival than any of the latter. In column 7 *b* of Table 8 there is a general tendency for the proportion of retarded larvae among the survivors to increase with increasing density, at least up to ten larvae per grain. When there was initially only one larva per grain 3·9% of the survivors had retarded rates of development, which shows that the phenomenon is at least to this extent independent of crowding. Now if it is assumed that a fixed percentage (e.g. 3·9%) of the larvae hatching have constitutionally retarded rates of development then, as the initial number of larvae per grain increases, the absolute number of retarded larvae will increase also. If the survival rate of the latter is greater than that of normal larvae then, since the number of larvae which survive per grain is limited, the proportion of retarded larvae among the survivors will increase. It is also possible, of course, that crowding is a cause of the retarded rate of development (although, as already stated, not always) and that this is the explanation of the results in column 7 *b*. Above ten larvae per grain perhaps the probability of elimination early in development is so great that the possession of an atypical rate of development is no longer an advantage.

There is no similar phenomenon in *Rhizopertha*. The dates of emergence of the adults emerging from one grain were separated by less than 7 days in 48 = 78% of the 62 grains from which more than one adult emerged, by 7–14 days in 9 = 14%, by 14–21 days in 2 = 3·2%, and by over 21 days in 3 = 4·8%. In the grains in which only one insect completed its development the adults emerged within 14 days of each other in 470 = 96·7% out of 486 grains. Furthermore, the proportion of retarded larvae was always very small.

Overcrowding in the larval instars seems to have had no other effect upon these insects than to increase mortality and to affect the numbers of larvae with retarded development in the way described above. The average developmental period of the majority of the larvae remained unaffected. The sex ratio of the emerging adults did not differ significantly from unity at any density for either species. The average weights of these adults at all densities did not differ significantly from the following values: *Rhizopertha* males, 1·17 mg.; females, 1·3 mg.; *Sitotroga* males, 1·8 mg.; females, 3·7 mg. The rates of oviposition in wheat of ten of the females which had developed at each density were measured at a density of two grains per female (Crombie, 1942, Tables 1 and 11). The average rates of oviposition for all these females were 9·8 eggs per female per day for *Rhizopertha* and 112 eggs per female per 5 days for *Sitotroga*. The rates of oviposition of each species at any density did not differ significantly from these values, respectively. The 143 grains from which one *Rhizopertha* emerged weighed 7·15 g. (50 mg. per grain) before and 5·50 g. after the insects had developed in them. The faeces and frass were removed from the still uneaten part of the grain before the second weighing. The loss in weight incurred through the development of one *Rhizopertha* per grain was therefore 1·65 or 23% of the original weight. The average amount of food eaten per larva during development (i.e. the average loss per grain) was 11·5 mg. This figure will be too high since some feeding would have been done by the adults before they ate their way out of the grains after emergence. The loss in weight of the 104 grains (5·2 g.) from which one *Sitotroga* emerged was 1·92 g. or 37% of the original weight. The average amount of food eaten per larva during development was therefore 18·5 mg. The average numbers of insects surviving per grain when $i > 1$ (1·16 for *Rhizopertha* and 1·1 for *Sitotroga*) are therefore less than the food present in a grain could support if unhindered by other forms of competition between the larvae (vide infra).

The effect of competition (with initially equal numbers of larvae) upon the survival of Rhizopertha *and* Sitotroga. The first experiment was a repetition of those just described except that larvae of the two species were present at the same time in equal numbers, i.e. an equal number of each species was competing for the same grain in each vial. The results are shown in Table 9 and Fig. 3.

The total number of insects emerging per grain (column 5, instar I) and the percentage of the total number of larvae surviving to become adults (column 7, instar I), respectively, do not differ significantly from the values of the same variables when either species was developing alone in the grains (Table 8). This means that the average value of a (equation (2)) here is really equal to those found in Table 8. There is no regular change in the value of a with larval density. The observed values of $100(1-P)$ shown in column 7, total, were compared with the values calculated with $a = 1{\cdot}21$ (not shown), and χ^2 revealed no significant difference between them. When each species is considered separately the average values of a are 0·69 for *Rhizopertha* and 0·52 for *Sitotroga*. The expected values of $100(1-P)$ were calculated with these values of a, respectively, and proved to be not significantly different from the observed values for each species, respectively, when tested by means of χ^2. This means that each species reduces the probability of survival of the other in direct proportion to its own numbers, and that there is at all densities a constant relationship between the numbers of the two species which survive. *Rhizopertha* was always more successful in competition than *Sitotroga*, the average ratio between them being *Rhizopertha* to *Sitotroga* as 57 : 43 = 1·3 : 1. (The value of χ^2 calculated in order to compare the numbers of adults of the two species in column 4 (instar I) corresponded to $p < 0{\cdot}01$, showing that the difference between the two sets of values is significant.) At larval densities above ten per grain this relationship may seem to change to the disadvantage of *Sitotroga*, but the agreement of observed and expected values of 100 $(1-P)$ argues against this, and furthermore with such small numbers the ratio between the survivors of the two species is very sensitive to random fluctuations. Approximately the same relationship is found when the larvae of both species were introduced in the second instar. The weight and sex ratio of the adults were, as before, unaffected by density, and were not significantly different from the values already given with each species developing in the grains separately.

Table 9. *The effect of density upon the survival of* Rhizopertha *and* Sitotroga *larvae competing for wheat. Each species initially present in equal numbers*

(1)			(2)	(3)			(4)			(5)			(6)		(7)			(8)	
Initial no. larvae per grain (i)			No. grains	Total no. larvae			Total no. adults emerging			Average no. adults emerging per grain			% of total adults comprised of		% larvae developing into adults $(1-P)\times 100$			% of survivors with development retarded by over 21 days	
R.	*S.*	Tot.		*R.*	*S.*	Tot.	*R.*	*S.*	Tot.	*R.*	*S.*	Tot.	*R.*	*S.*	*R.*	*S.*	Tot.	*R.*	*S.*
Instar I																			
1	1	2	75	75	75	150	42	32	74	0·56	0·43	0·99	57	43	56	43	49	2·3	6·25
2	2	4	70	140	140	280	46	41	87	0·66	0·59	1·25	53	47	33	29	31	2·2	27·0
3	3	6	70	210	210	420	54	40	94	0·77	0·57	1·34	58	42	27	17·6	22·5	3·5	32·4
4	4	8	60	240	240	480	41	35	76	0·68	0·58	1·26	54	46	17	14·6	15·8	2·4	28·5
5	5	10	50	250	250	500	38	35	73	0·76	0·70	1·46	52	48	15·6	13·8	14·6	2·6	44·0
7	7	14	30	210	210	420	22	9	31	0·73	0·30	1·03	71	29	11	3·7	7·4	0	0
10	10	20	30	300	300	600	24	8	32	0·80	0·27	1·07	75	25	8·3	2·3	5·3	0	14·3
Total			385	1425	1425	2850	267	200	467	0·69	0·52	1·21	57	43	19·2	13·6	16·4	2·0	29·5
Instar II																			
2	2	4	25	50	50	100	23	14	37	0·92	0·56	1·48	62	38	46	28	37	0	43

The effect of overcrowding upon the developmental period of the survivors was similar to that already described for each species separately. The percentages of the surviving insects with retarded development are shown in column 8. As before the number of adults emerging per grain (column 5, total) and the proportion of retarded *Sitotroga* (column 8) are highly positively correlated, the correlation coefficient being significantly different from zero at the 1% level, and positive. The proportion of retarded *Rhizopertha* was always very low, comprising only 2% of the total survivors, while that of retarded *Sitotroga* comprised 29·5% of them. The latter proportion is significantly higher than the proportion of retarded insects (3·9%) when there was initially only one *Sitotroga* per grain (Table 8 (χ^2 corresponds to $p < 0{\cdot}01$). Thus a large proportion of the *Sitotroga* which survive after competition for the same grain with *Rhizopertha* had retarded developmental periods. There were fifty-two grains in all from which individuals of both species emerged. The dates of emergence of the individuals of the two species developing in the same grain were separated by over 21 days in 15 = 29% of these grains, by 14–21 days in 26 = 50%, and by less than 14 days in 11 = 21%. The developmental periods of all the adults were within 10 days of the same length in 300 (= 90%) of the 333 grains from which one individual of either species emerged. There were 41 (79%) grains in which the developmental periods of the two species differed by more than 14 days. In 39 (i.e. 75% of the total 52 grains) of these the rate of development of the *Sitotroga* was retarded while that of the *Rhizopertha* was of the average value (28–38 days), and in only two (= 3·85%) did the opposite relationship hold. This suggests that the competition of *Rhizopertha* with *Sitotroga* larvae whose rates of development differ widely from their own is less severe than with those *Sitotroga* whose rates of development approximate their own. The proportion of retarded insects among the survivors of *Sitotroga* increases with increasing density up to ten larvae per grain, when the value of 44% is reached. As before there is a fall in this value with initially more than ten larvae per grain. It is clear that the possession of a proportion of larvae with atypical rates of development is of considerable survival value for *Sitotroga*, enabling more individuals of this species to survive in the limited space of one wheat grain, both when in competition with *Rhizopertha* and when only *Sitotroga* larvae are competing for the grains. The cause of retarded development in *Sitotroga* larvae is unknown.

The effect of non-contemporaneous entry into grains upon the competition of Rhizopertha *and* Sitotroga. The initial larval density was kept constant at four first instar larvae per grain (two of each species), but in one series of vials *Rhizopertha* was introduced 7, 14, 21 and 28 days before *Sitotroga* (Table 10 *a*), while in another series *Sitotroga* was introduced 7, 14, 21 and 28 days before *Rhizopertha* (Table 10 *b*). The results are shown in Table 10 and Fig. 4. Statistically significant differences were discovered by calculating the values of χ^2 or t, and on the basis of these the following statements are made (in each case $p<0·05$). (*a*) The relationship between the two species as observed by their survival (columns 5 and 7), relative to their relationship when both species were introduced at the same time (control), remained always to a greater or lesser extent in favour of the species introduced first (cf. Smith, 1912; Picard, 1922). (*b*) For the second species the most unfavourable interval at which to follow the first was 7–14 days. (*c*) As in previous experiments, however, the *Rhizopertha* were relatively more successful than the *Sitotroga*. For instance, while the survival of *Rhizopertha* was unaffected by *Sitotroga* which follow it by 14 or more days (column 5, and Table 8 *a*), *Sitotroga* always had a lower rate of survival when *Rhizopertha* followed it at any interval of time than when it was alone (column 5 *b*, and Table 8 *b*). (*d*) As the time interval between the introduction of the two species became longer (above 7 days), the number of adults emerging per grain of the first species eventually reached a fairly constant value, while that of the second species gradually increased (column 5). The total number of insects emerging per grain thus increased also. This was achieved by an increase in the number of wheat grains from which more than one adult emerged (column 6), which suggests that the mortality which occurred in the immature stages of the competing insects was not primarily due to lack of food. Additional evidence in support of this conclusion comes from two sources. The first is the observation that up to three and four normal-sized adults of either or both species have been known to emerge from a single grain. Secondly, as already mentioned, the average loss in weight of the wheat grains as a result

Table 10. *Effect of introducing one species at different periods of time before the other upon the competition of* Rhizopertha *and* Sitotroga *developing in wheat grains. Each species initially present in equal numbers*

(1) 1st instar larvae of one species introduced *x* days before those of the other	(2) No. grains	(3) Total no. larvae (2 *R.*+2 *S.* per grain)			(4) Total no. adults emerging			(5) Average no. adults emerging per grain			(6) No. grains from which both species emerged	(7) % of total adults comprised of		(8) % larvae developing into adults		
		R.	*S.*	Tot.	*R.*	*S.*	Tot.	*R.*	*S.*	Tot.		*R.*	*S.*	*R.*	*S.*	Tot.
Control: both introduced at same time (Table 9)	70	140	140	280	46	41	87	0·66	0·59	1·25	8	53	47	33	29	31
(*a*) *Rhizopertha* before *Sitotroga*																
7 days	30	60	60	120	24	7	31	0·8	0·23	1·03	0	78	22	40	11·7	25·7
14 ,,	30	60	60	120	34	13	47	1·13	0·43	1·56	12	83	17	56·7	21·7	39·3
21 ,,	30	60	60	120	31	24	55	1·03	0·8	1·83	17	56	44	51·7	40	45·7
28 ,,	30	60	60	120	36	21	57	1·2	0·7	1·9	21	63	37	60	35	47·5
(*b*) *Sitotroga* before *Rhizopertha*																
7 days	30	60	60	120	10	25	35	0·33	0·83	1·16	5	29	71	16·7	41·7	29·3
14 ,,	30	60	60	120	16	25	41	0·53	0·83	1·36	11	37	63	26·7	41·7	33·3
21 ,,	30	60	60	120	23	26	49	0·77	0·87	1·64	16	47	53	38·3	43·3	40·1
28 ,,	30	60	60	120	25	26	51	0·83	0·87	1·7	16	49	51	41·7	43·3	42·5
(*c*) The larvae of both species were introduced at the same time in the instars shown																
R. *S.*																
II I	20	40	40	80	20	12	32	1·0	0·6	1·6	9	63	37	50	30	40
III I	20	40	40	80	20	18	38	1·0	0·9	1·9	16	53	37	50	45	47·5
I II	20	40	40	80	9	15	24	0·45	0·75	1·2	2	38	62	22·5	37·5	30
I III	20	40	40	80	14	15	29	0·7	0·75	1·45	12	48	52	35	37·5	36·3

of the successful development of one insect per grain is only 23 % of the original weight of the grain for *Rhizopertha* and 37 % for *Sitotroga*. Now the species introduced first eats food, uses up oxygen and conditions the inside of the grain with its faeces and other waste products. However, if these were the principal causes of the results one would expect that the longer the interval between the introduction of the two species the greater would be the depression of the species introduced second. This was not so, so that the principal cause of the results must have been the active competition of the larvae inside the grains for space (cf. Pemberton & Willard, 1918; Lloyd, 1940).

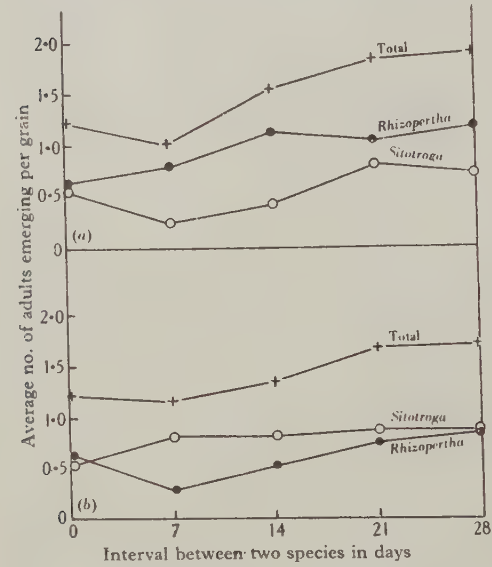

Fig. 4. The effect of introducing one species into the grains at different periods of time before the other upon the survival and competition of *Rhizopertha* and *Sitotroga* larvae (Table 10, column 5). (*a*) *Rhizopertha* introduced first, (*b*) *Sitotroga* introduced first.

On the other hand, 28 days after introduction the first species would usually be in the pupal instar. It is therefore not likely to have engaged in active competition. Now the food provided by one grain is as already mentioned adequate for the complete development of at least two larvae. The reason why the second species survived better in an otherwise uninhabited grain (Table 8) than in a grain in which the first species was preceding it by 21 days or more, is therefore likely to be that the first species conditioned the inside of the grain into which the second species enters. A considerable amount of faeces and frass was always made by the larvae (vide infra). (*e*) Sex ratio and weight of neither species was affected by competition.

Now in all except two of the 98 grains (column 6) from which both species emerged the interval of time between the emergence of the individuals of each species was greater than 21 days. As already mentioned, as the interval of time between their introduction becomes longer (above 7 days), there is a statistically significant increase in the number of grains from which both species emerged (column 6). Since the rates of development of the majority of individuals of both species would be approximately equal, the members of each species would be in more widely different instars as the interval of time separating their introduction increases. The results therefore agree with those obtained in previous experiments (Tables 8, 9), in which it was found that the mortality of *Sitotroga* and its competitors which were developing in the same wheat grain was greater among insects with approximately contemporaneous development than in those whose developmental periods differed by a great enough margin (21 days).

Why should the species introduced first have a relative advantage over the other species, and why should it be more unfavourable for the second species to be introduced 7–14 days after the first than either at the same moment or after longer intervals of time? The following experiment was performed in order to answer these questions. Two larvae of each species were introduced at the same time into each grain, but in each experiment the larvae of one species were in a more advanced instar than those of the other, as shown in Table 10 *c*. The numbers involved are rather small, but the results are remarkably similar to those shown in Table 10 *a*, *b*, the initially more mature larvae here bearing the same relationship to their competitors as those introduced first in the previous experiment bear to theirs. The mortality of the insects introduced in the first instar was in both species greater when the other competing species was introduced in the second instar than when the latter was introduced in the first or third instar. Now in both species the second instar occupies the period from about 6–12 days after hatching, so that in the previous experiments the larvae introduced first by 7–14 days would be in the second or third instar when the second species was introduced. Those introduced first by 21 days would be in the third or fourth instar by the time the second species was introduced. Furthermore, both the total number of insects emerging per grain, and the number of grains from which both species emerged, were greater when one species was introduced in the first instar and the other the third instar, than when one was introduced in the first instar and the other in the second instar, or both in the first instar. It seems reasonable to suggest that these differences in the severity of competition between larvae of different ages may be the explanation of the greater survival of larvae whose development was not contemporaneous with that of their competitors than of those in which it was (Tables 8, 9). As the larvae enter the grains after increasingly different periods (Table 10) the time during which they are in competition of course becomes shorter, but this does not explain why one species should suffer more when introduced days after, than at the same time as, the other. If the latter result is correct, it must be concluded then that the toleration of the larvae for each other at any moment varies with their relative ages. There is, however, another possible explanation. As mentioned above, *Rhizopertha* adults remain inside the grain for a few days after emergence before eating their way out. It is possible that these adults may damage other insects in the same grain. If the latter were following them by only a short interval they would be more likely to be in the pupal stage when these adults emerged than in the larval stage, and perhaps pupae are more susceptible to such damage than larvae. On several occasions dead and partly eaten pupae were found in grains from which other insects had previously emerged as adults, but the data were too meagre to be able to draw any definite conclusions. This explanation would not of course apply to the results obtained when *Sitotroga* was the first species introduced into the grains.

The effect of inequality of initial numbers upon the competition of Rhizopertha *and* Sitotroga. One *Sitotroga* first instar larva with increasing numbers of *Rhizopertha* first instar larvae were introduced into each grain in one series of vials (Table 11 *a*), while one *Rhizopertha* first instar larva with increasing numbers of *Sitotroga* first instar larvae were introduced into another series of vials (Table 11 *b*). The results are shown in Table 11 and Fig. 5. The total number of adults of both species emerging per grain and the percentage of the total larvae developing with adults are approximately equal to the values of these variables at comparable densities in Tables 8 and 9. More adults of the initially more numerous species always emerged per grain than of the initially less numerous species but, as in Table 9, *Rhizopertha* was more successful in competition than *Sitotroga*. The number of *Rhizopertha* which survived when it was in a minority is significantly greater than the number of *Sitotroga* which survived when it was in a minority (χ^2 corresponded to $p<0{\cdot}01$). The percentages in column 8 were calculated on the assumptions that the insects will be eliminated at random (i.e. that the probability of any insect surviving is inversely proportional to the initial number present) but that the probability of survival for *Rhizopertha* compares with the probability of survival for *Sitotroga* as 57 : 43 = 1·3 : 1. The latter is the average ratio of the survivors belonging to each species when there were initially equal numbers of larva of each species in each grain (Table 9). Let the initial number of *Rhizopertha* equal *r*, and of *Sitotroga* equal *s*. The probability that one *Rhizopertha* will survive is then equal to

$$r \div \left(r+\frac{s}{1\cdot 3}\right)=\frac{1\cdot 3r}{1\cdot 3r+s}.$$

Table 11. *Competition between* Rhizopertha *and* Sitotroga *developing in wheat grains with different initial numerical relationships*

(1) Initial no. 1st instar larvae per grain			(2) No. grains	(3) Total no. larvae			(4) Total no. adults emerging			(5) Average no. adults emerging per grain			(6) % survivors (observed) comprised of		(7) % larvae developing into adults			(8) Expected % survivors comprised of	
R.	*S.*	Tot.		*R.*	*S.*	Tot.	*R.*	*S.*	Tot.	*R.*	*S.*	Tot.	*R.*	*S.*	*R.*	*S.*	Tot.	*R.*	*S.*
Control (Table 9)																			
1	1	2	75	75	75	150	42	32	74	0·56	0·43	0·99	57	43	56	43	49	57	43
(*a*) *R.* > *S.*																			
2	1	3	80	160	80	240	58	24	82	0·73	0·3	1·03	71	29	36	30	34	73	27
4	1	5	80	320	80	400	75	17	92	0·94	0·21	1·15	82	18	23·5	21	23	84	16
8	1	9	80	640	80	720	70	11	81	0·87	0·14	1·01	86·4	13·6	11	13·8	11·2	91·5	8·5
16	1	17	80	1280	80	1360	69	9	78	0·86	0·12	0·98	89·4	10·6	5·4	11·2	5·7	95·5	4·5
(*b*) *R.* < *S.*																			
1	2	3	80	80	160	240	36	48	84	0·45	0·6	1·05	43	57	45	30	35	40	60
1	4	5	80	80	320	400	18	66	84	0·23	0·82	1·05	21	79	25	20·5	21	25	75
1	8	9	80	80	640	720	19	67	86	0·24	0·84	1·08	22	78	24	10·4	12	14	86
1	16	17	80	80	1280	1360	20	65	85	0·25	0·81	1·06	24	75	25	5·1	6·3	8	92
Total (*a*)+(*b*)			715	1360	1360	2720	407	339	746	—	—	—	54·9	45·1	—	—	—	—	—

Similarly, the probability that one *Sitotroga* will survive is $\frac{s}{1{\cdot}3r+s}$. The relative probability of survival is therefore *Rhizopertha* to *Sitotroga* as $1{\cdot}3r : s$. The agreement between expected and observed values was tested by calculating the value of χ^2, and there proved to be no significant difference between them.

There were only 18 grains from which both species emerged in this experiment; in 11 of these the *Sitotroga* emerged more than 21 days after the *Rhizopertha*. As before the weight and sex ratio of the insects remained unaffected by crowding.

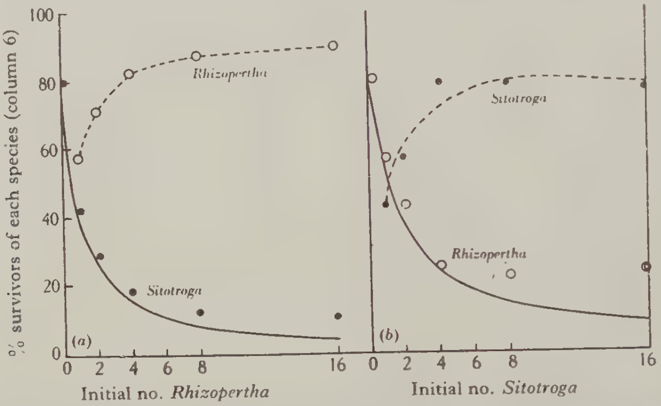

Fig. 5. The effect of the initial numerical relationship between them upon the survival and competition of *Rhizopertha* and *Sitotroga* larvae developing in wheat (Table 11, column 6). (*a*) *Rhizopertha* in a majority, (*b*) *Sitotroga* in a majority. The full line is drawn through the theoretical points (column 8).

V. SUMMARY

The females of both *Rhizopertha* and *Sitotroga* oviposit in environments containing places suitable for larval development, but the larvae themselves, usually during a period of migration in the first instar, choose the actual developmental site. The rate of oviposition of neither species of female bears much relation to the amount of food present for the larvae, and the latter do not refrain from multiple or superinfestation of wheat grains. The competition which ensues is apparently wholly a struggle for space, the limitation of food or oxygen, and the 'conditioning' of the medium, being unimportant. Larvae (of any instar) will attack each other directly after encounters at random within wheat grains, and the supernumerary individuals are either killed or forced to migrate. The probability that any particular larva will survive is thus inversely proportional to the initial number present (equation (1)). Except that it tended to favour the survival of *Sitotroga* with atypical rates of development, overcrowding had no other effect upon the larvae of either of these species. In an unlimited or unsaturated environment migration may lead to survival; but when the environment is limited in capacity a point will be reached when migration from one grain to another merely leads to death in another place. Then the only effect of overcrowding will be to increase mortality. Migration from the grains tends to decrease with later instars. Because of competition for space the number of larvae of the same age which survive in one grain is less than that which the food present in the latter could support. This number (approx. 1·2 per grain) does not vary with density, so that the relationship between survival and larval density is given by equation (2).

When the two species are competing the average ratio of the survivors is *Rhizopertha* to *Sitotroga* as $1{\cdot}3r : s$, where r and s, respectively, are the initial numbers of larvae of each species. These represent the proportions of the 1·2 larvae surviving per grain which belong to each species. This ratio remains constant at all densities when the larvae enter the grains at the same time in the same instar. Each species thus decreases the probability of survival of the other in direct proportion to its own numbers.

When first instar larvae of the two species enter the grains at different times the above relationship changes in favour of the first comer. The most unfavourable time for the second species to enter the grain is apparently when the first is in the second or early third instar. With greater differences between times of entry (i.e. of age) the severity of competition for space decreases, so that more larvae are able to survive and take advantage of the food reserves of the grain. (The survival of the larvae of the second species is apparently then reduced to some extent by the accumulation of the excretory products of the first.) *Sitotroga*, but not *Rhizopertha*, was able to take advantage of this decreased competition because of the occurrence of larvae with atypical rates of development in this species. The latter were able to survive the competition of normal larvae of either species where other normal larvae would have succumbed. Crowding to a certain degree tended to increase the proportion of atypical larvae among the survivors of this species.

Overcrowding in the immature stages had no effect upon the average developmental period of the larvae, or upon the sex-ratio, weight or fecundity of the adults or either species.

I should like to thank Dr A. D. Imms, F.R.S., and Dr W. H. Thorpe for their interest in these experiments and Dr A. R. Miller for his mathematical advice. This work was done while holding a research scholarship from the University of Melbourne.

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THE BIOLOGY AND BEHAVIOUR OF *PTINUS TECTUS* BOIE (COLEOPTERA, PTINIDAE), A PEST OF STORED PRODUCTS

V. HUMIDITY REACTIONS

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(*Received* 30 *November* 1943)

(With Eight Text-figures)

I. INTRODUCTION

This paper continues the series on *Ptinus tectus* (Bentley, Gunn & Ewer, 1941; Gunn & Hopf, 1942; Ewer & Ewer, 1942; Gunn & Walshe, 1942). The correlation to be observed between moisture conditions and the intensity of insect infestations of stored products' needs no emphasis. For this reason it is desirable to analyse as far as possible the humidity reactions of such a well-established pest as *P. tectus* has become in this country. Although the species is not ideal for an attack on the outstanding problems of the humidity reactions of insects, the results obtained contribute to the growing body of knowledge in this field.

II. APPARATUS AND METHODS

The apparatus used in the majority of experiments was the *alternative chamber* or choice chamber of Gunn & Kennedy (1936) as modified by Bentley *et al.* (1941). This consists essentially of a shallow glass dish, 22 cm. in diameter, with a false floor of perforated zinc and a glass lid sealed by vaseline. Under the false floor are placed Petri dishes of the required strengths of sulphuric acid and the Edney paper hygrometers. In a few experiments a *ring gradient*, 90 cm. in diameter, was used (Gunn & Kennedy, 1936). In this, a circular track on perforated zinc is available to the animals, the gradient being controlled by granular calcium chloride and water and measured in the same way as before. All the work was done at a constant temperature of 25 ± 0·5° C. and under constant light from a high ceiling lamp which gave an approximate intensity of 20–30 m. candles at table level.

In the experiments on preferred humidity ten animals were used in each choice chamber. A zone, 1 cm. wide, divided the gradient into a drier part and a wetter part. After each reading the animals were moved by means of a pipe-cleaner into the middle of the dish, or so distributed that there were equal numbers on each side of the middle zone. A preliminary experiment showed that the maximum humidity reaction did not occur until 3 or 4 hr. after the animals had been dropped into the choice chamber; during this period they showed more activity than they did later. After this, maximal intensity of reaction occurred within 1 hr. of stirring. Accordingly, in all experiments the first observation was made $3\frac{1}{2}$ hr. after the animals had been put into the choice chamber and subsequent observations at hourly intervals. Using this method, eight readings were made in a day. For a number of reasons it was not found possible to follow the usual practice of repeating each experiment on the following day with the acid dishes and hygrometers reversed.

The index used to express intensity of reaction may be represented as $\{100\,(D-W)\}/N$, where D represents the number of position records on the drier side, W the number of the wetter side and N the total number of observations (that is, including animals in the middle zone). The advantages of this index are that it gives equal prominence to 'wet' and 'dry' reactions and it takes into account those animals which were in the middle zone at the time of observation.

In the experiments on locomotory activity at different constant humidities, ten animals were used in each choice chamber. In recording, a distinction was made between itinerant or active beetles and beetles which were 'virtually inactive', that is, showing movements which, if continued, would not carry them to another part of the chamber (Pielou & Gunn, 1940). Observations were made by the cross-section method (Bentley *et al.* 1941) in which the observer chose a random instant at which to note the behaviour of the insects.

All animals were bred at 25° C. in constant illumination on wholemeal flour to which 5% of dried baker's yeast had been added. A supply of water maintained a R.H. of about 70% and also functioned as a drinking supply. Unless otherwise stated, beetles were removed from cultures on the day before an experiment and transferred to wet cotton-wool from which they could make up any water deficiency. After 15 min. a wad of dry cotton-wool was introduced. The beetles were picked off this on the following day and put in the experimental dishes. The dishes were set up on the previous afternoon and allowed to equilibrate overnight.

As at present the only way to obtain large numbers of *P. tectus* of the same sex is extremely laborious, the insects used were not chosen for sex. As far as possible beetles of approximately the same age were employed, ranging from 2 to 5 weeks old.

The experiments on the location of the receptors involved amputation of varying numbers of antennal segments. It was found unnecessary to anaesthetize. With practice it became possible to remove the required number of segments with a pair of iridectomy scissors after turning the beetle on its back and teasing with a fine paint brush until it gave a shamming reaction. Individuals which bled much were rejected, but they were very few in number. In these experiments the animals, together with their controls (which were subjected to approximately the same amount of handling), were kept in a damp atmosphere for 2 days before experiment.

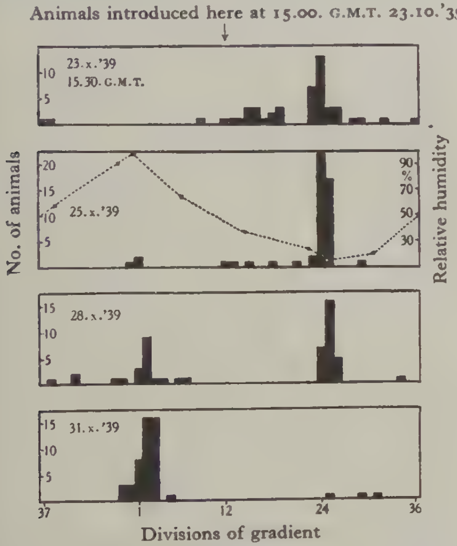

Fig. 1. Results from an experiment in the ring gradient. Fifty *Ptinus tectus* were watered and introduced as shown. After about a week the aggregation near the calcium chloride was replaced by a preference for wet air. The gradient remained steady throughout.

III. RESULTS

1. *Experiments with the ring gradient*

To gain a rough idea of the nature of the humidity preference of *P. tectus*, fifty insects were allowed to make up any water deficiency and introduced into the ring gradient mentioned above. The apparatus gave a range extending from 14·5 to 98·0% R.H. representing a gradient of 0·6% R.H. per cm. It remained very constant throughout the experiment.

Fig. 1 shows that within 30 min. there was a considerable aggregation in the dry region. This became more pronounced and then suffered little change for 5–6 days. The beetles forming the aggregation remained in a dense clump from which single animals would detach themselves temporarily, only to return after a few minutes. Towards the end of a week the insects began to disperse and to migrate up the gradient. After 8 days from the start of the experiment 94% of the beetles were in the immediate vicinity of the water. It was noticeable that the animals no longer clumped but formed much looser aggregations and were restless. At this stage one insect was dead. The remainder were removed from the gradient and separated into five groups. Each group was weighed, allowed to drink, dried and reweighed. The gain in weight was reasonably consistent, varying from 17 to 29% and averaging 22% of the desiccated weight. Most of the beetles were in air of about 16% R.H. for the greater part of the experiment, and the average rate of loss of water (approx. 2½% per day) indicates considerable powers of resistance to desiccation (cf. 4% per day at 25° C and 0% R.H. in the case of *Tenebrio molitor* (Pielou & Gunn, 1940). In another similar experiment it was noticed that after the desiccated animals had been watered and put back in the gradient they usually, but not invariably, went *immediately* to the region of the calcium chloride as at the beginning of the experiment.

2. *The effect of humidity level and desiccation on locomotory activity*

The previous experiment indicated that the humidity preference of *P. tectus* depends largely on the degree to which the insect has lost water. Before proceeding to a more detailed analysis of the behaviour by means of choice-chamber tests, two sets of experiments were carried out to determine the effect of desiccation on locomotory activity in the absence of humidity gradients. The two sets yielded similar results, but the second only will be discussed.

Twelve lots of ten animals were watered and kept overnight at 70% R.H. The following day they were transferred to experimental chambers in which a constant humidity was maintained. The humidity levels chosen were 0, 30, 80 and 95%, and there were three experiments at each level. Eight hourly observations were made each day from 0930 to 1630 G.M.T. Fig. 2 shows the mean activity (i.e. excluding virtual inactivity) for the three experiments at each humidity. Each point represents an average for two consecutive days (sixteen observations). For the first 12 days they are based therefore on 480 records. After this period some of the insects were dead. The total number remaining alive (out of thirty) at each humidity is shown on the graph.

Fig. 3 shows the 2-day averages for the individual experiments at 0% R.H., and indicates a fair degree of correspondence in the results obtained. It is clear that humidity level has a well-defined effect on locomotory activity. For the first week there is no significant difference between the activity at 0 and that at 30 % R.H. At 80 % R.H., however, the activity observed is about double what it is at the lower humidities, while at 95 % R.H. it is approximately four times as high. Of particular interest is the behaviour of the insects at 0 % R.H. and therefore subject to the greatest desiccation. At the end of a week the activity of these animals increased, till after 11–12 days it was greater than even that of the beetles kept at 95 % R.H. A few days later nearly half had died and several of the remainder were obviously moribund. A similar increase in activity took place in the case of the insects at 30 % R.H. but at a correspondingly later stage of the experiment.

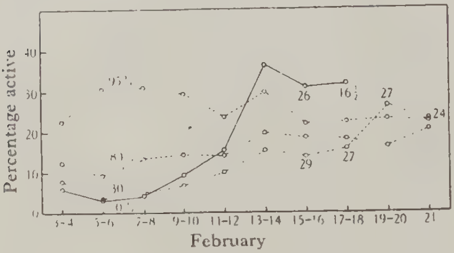

Fig. 2. Two-day averages from four sets of three experiments on the effect of humidity on the locomotory activity of *Ptinus tectus*. The values opposite certain of the points indicate the number of animals surviving out of thirty. Each of the other points represents 480 records.

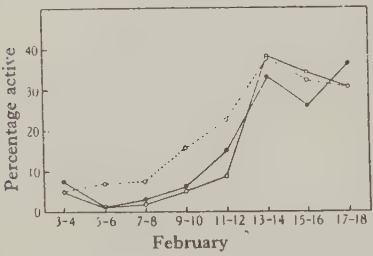

Fig. 3. Two-day averages of three experiments on the locomotory activity of ten *Ptinus tectus* at 0 % R.H. Eight hourly observations were made each day.

These results are in agreement with those obtained in the experiments with the ring gradient. The low activity at first at 0 % R.H. compared with that at 95 % R.H. suggests that the aggregation at the dry end may be due to a simple kinetic mechanism. That the humidity behaviour of *P. tectus* is more complex than this is indicated by results of the choice-chamber experiments presently to be described. The increase of activity with desiccation is also consistent with the alteration of preference shown by the ring-gradient animals under similar conditions. The results of both sets of experiments stress the importance in humidity tests of choosing animals in which the degree of desiccation has been carefully controlled.

3. *The humidity responses of watered beetles*

Approximately 100 experiments were carried out in the choice chamber in an attempt to analyse the stages leading up to the final reaction observable in the ring gradient. The variation in the results obtained in preliminary experiments indicated the advisability of concentrating on small differences of humidity over six or seven different regions of the scale. Thus, in the majority of the experiments, the acid differences used were 0–15, 15–30, 30–45, 45–60, 60–75, 75–90 and 90–100 % R.H. These gave actual humidity gradients of approximately 11 % R.H. except at the wet end, where the average lay between 94 and 99 % R.H. To avoid hysteresis effects in the hygrometers the calibration of the instruments used in dry air was never carried below 5 % R.H., and the lowest humidities were therefore estimated by extrapolation.

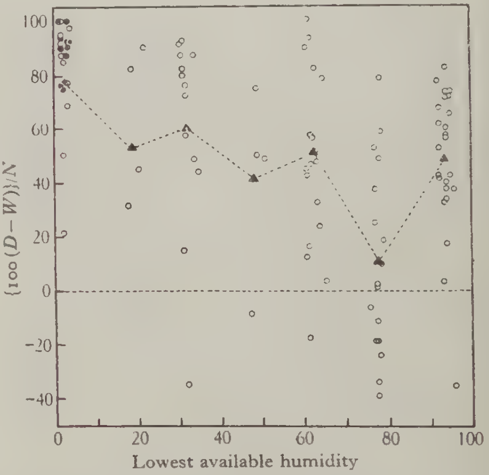

Fig. 4. The humidity preference of recently watered *Ptinus tectus*. Intensity of reaction is expressed in terms of the excess percentage of animals on the dry side and is plotted against the lowest humidity available. The ranges were 10·3–12·2 % difference of humidity (5 % at the 90 % R.H. range), with the exception of ten experiments (indicated by blocked-in circles) in which the range available was approximately 18 % R.H. The dotted line joins up the mean intensities of reaction.

Fig. 4 shows the values obtained for the mean intensity of reaction (in terms of excess percentage on the dry side of the gradient) in each of the experiments performed. Each point is derived from eighty position records extending over a period of 8 hr. Ten experiments were carried out with the steeper gradient obtained by using acids subtending atmospheres of 0 and 25 % R.H. These results are also indicated in Fig. 4. There were not enough of these experiments to show whether the lowest or highest humidity available is the more significant in determining the intensity of the reaction. In the present analysis the intensity is plotted against the lowest R.H. recorded.

The kind of variation in intensity of reaction to humidity revealed by *P. tectus* in different regions of the scale is unique when compared with the behaviour of all other animals studied. Ignoring the considerable individual variation for which no explanation is offered, it is plain that on the whole the insects show a preference for dry air. This preference is particularly intense both at the drier end of the gradient and in very damp air. In air in which there is a gradient between about 77 and 89 % R.H. there is no significant reaction either one way or the other.

In the light of these results, whatever the underlying mechanism of the reaction, the aggregation at the dry end of the ring gradient might be expected. Insects at the wet end would tend to migrate into the region of no reaction. Any return to the wet end resulting from the appreciable activity already noticed at this R.H. level would not be permanent. On the other hand, a similar undirected wandering into a region below the zone of no reaction would tend to be continued towards the drier end. One might expect therefore to find a loose secondary aggregation in the 75–90 % R.H. region in the early stages of the ring-gradient experiments. This has not been observed, possibly owing to the masking effect of the abnormally high activity which *Ptinus* invariably exhibits after handling.

The effect of steepness of gradient was not studied specifically, but the results of the above-mentioned experiments in the 0–25 % R.H. range can be compared with those obtained from the ten experiments in the 0–15 % R.H. range. In the first series the mean gradient used was 3·0–21·0 % R.H. (a difference of 18·0 % R.H.) and the mean I.R. was 92·0 ± 8·5 %. The less steep gradient averaged 2·5–14 % R.H. (a difference of 11·5 % R.H.) and the mean I.R. was 77·7 ± 24·9 %. Statistical analysis shows that the observed difference between these two means is insufficient to indicate a clear correlation between intensity of reaction and the steepness of the gradient offered.

4. *The reactions of desiccated beetles*

In view of the reversal of humidity preference which eventually occurred in the ring gradient, the experiments referred to in the previous section were repeated on a smaller scale with insects which had been kept without water for several days. Two sets of experiments were carried out using the following acid ranges: 0–15, 75–90 and 90–100 % R.H. Desiccation was brought about by keeping the beetles for 10 days at 0 % R.H. They may thus be compared with the insects used in the experiment on the effect of 0 % R.H. on activity level, at a time 10 days after the experiment had begun and when activity had become quite high (see Figs. 2, 3). They may also be compared with the ring-gradient insects after these had migrated to the wet end of the apparatus (Fig. 1). Two types of control were used. In the first series of experiments the control animals were desiccated with the experimental animals until a few hours before observations began and then they were given water. In the second series they were watered normally throughout the 10 days before the experiments. Fig. 5 shows the results obtained, each point on the graph representing, as before, eighty position records.

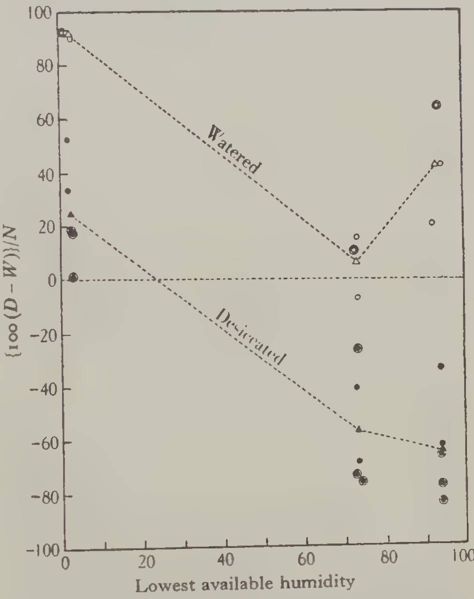

Fig. 5. Two sets of experiments on the humidity preference of desiccated *Ptinus tectus* in three gradients. The ordinates are the same as in Fig. 4. ○ controls, 1st set of experiments. ⊙ controls, 2nd set of experiments. △ mean of both sets. ● desiccated beetles, 1st set of experiments. ⊙ desiccated beetles, 2nd set of experiments. ▲ mean of both sets.

The two series of experiments gave essentially the same results. In each case the values obtained for the controls compare well with the mean intensities of reaction established in the experiments described in the previous section (Fig. 4). It may be inferred that, whatever the changes in the nature of the humidity response which occurred in the desiccated insects, they were reversed on drinking. It is justifiable, therefore, to combine the results from the two sets of control experiments. This has been done in Fig. 5.

The desiccated beetles behaved very differently from beetles which had recently drank. In the 0–15% R.H. range the reaction was still apparently towards the drier side though reduced in intensity. In the other two ranges the 'dry' reaction was replaced by a reaction towards the wetter end of the gradient. This result is not inconsistent with the migration to the 'wet' zone shown by the ring-gradient insects after desiccation, for, whereas the slight 'dry' reaction in the lowest humidity range would have a trapping effect, this would be offset by the enormous increase in activity which occurs with desiccation (Fig. 2). Such an increase of activity might easily take the insect outside the range where the 'dry' response is effective.

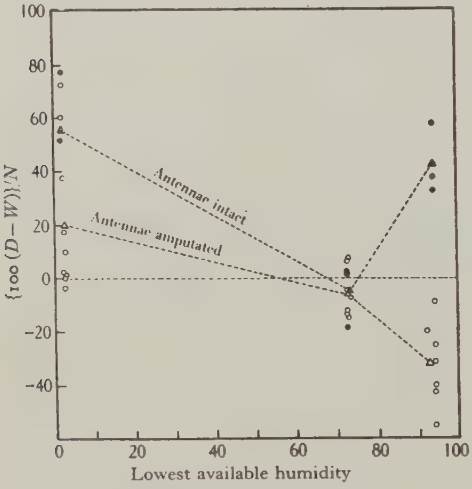

Fig. 6. The humidity preference of antennae-less *Ptinus tectus* in the same gradients as were used in the experiments shown in Fig. 5. Blocked-in symbols indicate control experiments; triangles indicate the mean intensities.

5. *Experiments on the location of the humidity receptors*

Possible interpretations of the reversed reaction to humidity shown by *Ptinus* when it is desiccated may depend on the nature of the receptors. An attempt was therefore made to locate them. The information obtained, though incomplete, may be of interest as far as it goes. The antenna of *P. tectus* consists of eleven segments, of which the basal and the most distal are rather larger than the other nine. In the experiments to be mentioned, extirpations of various numbers of segments were carried out. The operations were always bilateral and in no case was the basal segment removed as it was considered inadvisable to operate too near the brain. Preliminary experiments showed that, though the removal of segments apparently did not affect the proportion of the day during which the animals moved about, the operated beetles moved at a much slower speed than their controls. As reaction to humidity and activity level are usually closely linked it is therefore inadvisable to draw comparisons between strengtbs of response of de-antennate and antennate insects. Fig. 6 shows the results of a number of choice-chamber experiments on watered beetles in which ten of the eleven segments of the antenna had been removed. The three humidity ranges used were the same as in the experiments on desiccated animals. In the 0–15% R.H. range the preference was still 'dry' but less than that shown by the controls. In the 75–90% R.H. range there was no significant reaction. In very damp air, however, the operated beetles behaved very much like unoperated, *desiccated* beetles, giving a strong 'wet' reaction.

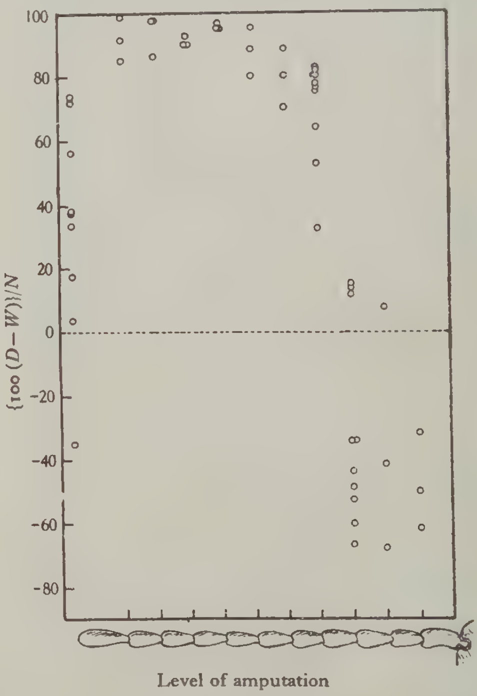

Fig. 7. The humidity preference of *Ptinus tectus* between 94 and 99½% R.H. following bilateral extirpation of antennae at various levels. The control experiments are shown to the left.

This result led to some fifty further experiments in the 90–100% R.H. range with beetles lacking various numbers of antennal segments. Fig. 7 shows the results obtained. When the terminal segment was removed an increased preference for dry air occurred. This persisted with successive extirpations till six segments had been removed. Thereafter the intensity of the dry reaction fell off rapidly until with only three segments remaining the reaction was predominantly towards the wetter air. Interpretation of the results of these two sets of experiments will require further experiment, especially in view of the reduced, but significantly, dry reaction shown by antenna-less beetles in the 0–15% R.H. gradient. They do suggest, however, the possibility of two sets of receptors being involved, of which one set is mainly confined to the distal eight segments of the antenna. Examination of sections and whole mounts failed to reveal among the various structures present any which either suggested a humidity-receptive function or which corresponded in distribution to that suggested above (Fig. 8). The antenna of *P. tectus* bears, for example, nothing resembling the humidity receptors described by Valentine (1931) and identified by Pielou (1940) for *Tenebrio molitor*, sections of the antenna of this insect being cut by the same technique for the purpose of comparison. Nor are there any structures on the *antenna* resembling the humidity receptors identified for the louse, *Pediculus humanus*, by Wigglesworth (1941).

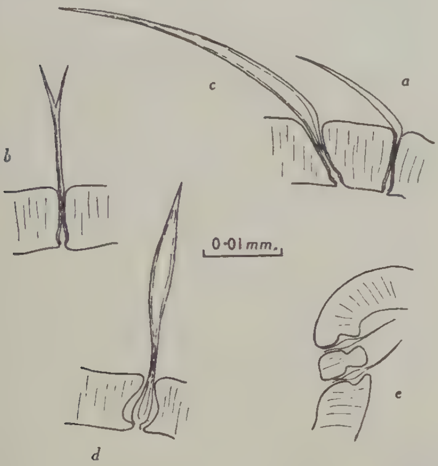

Fig. 8. Structures found on the antennae of *Ptinus tectus*. (*a*) short bristles abundant on distal segments; (*b*) forked bristles most abundant on proximal segments and on head; (*c*) coarser bristles also more frequent on proximal segments; (*d*) spear-shaped bristles occurring in large numbers only on terminal segment; (*e*) pit-hairs from rounded knob on which basal segment is set.

IV. DISCUSSION

In spite of the considerable variation in intensity of response to humidity, when *Ptinus tectus* has had access to water to drink, it generally aggregates in the driest region of a gradient. The tendency is not, however, equally apparent over all humidity ranges and is usually absent between 75 and 90% R.H. No explanation for this latter fact is offered. There is no reason to expect that an insect's receptors are equally sensitive over all ranges of stimulation, more particularly in this case where it is difficult to see what advantages would accrue from being able to differentiate between regions of a gradient which is probably equally favourable over its whole extent.

The experiments described above also show that as desiccation proceeds the reaction towards drier air disappears and is replaced by a reaction towards regions of higher humidity. Furthermore, the change in direction of response is accompanied at approximately the same point in time by a considerable increase of activity. The biological advantages of this are obvious.

Reactions towards dry air have been recorded for other insects. Kennedy (1937) noted a small but definite dry reaction over the whole of the scale in his experiments on the gregarious phase of *Locusta migratoria*. Here the intensity of reaction depended on the difference of humidity available. When this was less than 20% R.H. the reaction approached zero.

Less marked dry reactions were also found by Thomson (1938) in *Culex fatigans*, Gunn & Cosway (1938) in *Blatta orientalis*, and Pielou & Gunn (1940) in *Tenebrio molitor*. As pointed out by Kennedy, all these reactions result in aggregation in air the humidity of which might be considered as unfavourable. *Locusta* develops best between 60 and 75% R.H. (Hamilton, 1936); *Ptinus tectus* breeds best at about 70% R.H. (Ewer & Ewer, 1942); Thomson (1938) states that hungry females of *Culex fatigans* do not avoid humidities that are fatal. It would seem then that this preference for dry air invites comment. It must be remembered, however, that in the field the air humidity may never be as unfavourably low as it is in these experiments. Further, even when low humidities occur, it probably happens that other physical agencies combine to prevent the dry reaction taking place. As shown by Bentley *et al.* (1941), *Ptinus tectus* is most active at night. Temperature is then lowest and humidity highest for the day. The beetle's photonegative behaviour would also tend to keep it in a sheltered and therefore probably damper environment during hours of sunlight and high temperature.

The reversal of direction of response on desiccation is an added protection against prolonged exposure to dry atmospheres. Gunn & Cosway (1938) in their studies of the cockroach have also recorded a slight wet reaction after desiccation. The point may be raised as to whether *Ptinus* is often able to obtain liquid water in the field or whether it maintains its water balance by utilizing the water content of its food. Observations on the distribution of the insect in warehouses and stores in this country, as distinct from its original natural environment, suggest that the latter may usually be the case and that it lives permanently on the verge of desiccation.

From the economic viewpoint this may be fortunate, for Ewer & Ewer (1942) have reported a rise in the rate of oviposition in laboratory beetles which had been given water to drink. Their results are supported by the observation occasionally made that *P. tectus* shows a higher incidence in situations where food has become damaged by water, though it is true that this may be related to other factors such as the optimal humidity for larval development. In any case it is suggested that the humidity reactions of beetles found as pests of stored products in this country may be less markedly towards drier air than those of watered insects bred in the laboratory. Experiments begun with a view to deciding this point were unfinished when the work had to be suspended.

V. SUMMARY

1. *Ptinus tectus* adults react to a humidity gradient by collecting in the drier region.

2. The reaction to a given difference of humidity is most intense at low humidities; it declines to nothing at about 75–90% R.H. and increases again at 90–100% R.H. No such variation has previously been found.

3. A kinetic mechanism of reaction is involved. The higher the humidity the higher the locomotory activity over a period of a week.

4. Desiccated animals are more active than normal ones. At low humidities they show a much weakened reaction towards drier air; at high humidities the normal reaction is reversed and the animals collect in the wetter region.

5. Desiccated animals given water to drink immediately begin to behave like normal ones.

6. Some of the humidity receptors appear to be located on the antennae. Removal of the distal joint increases the intensity of reaction in high humidities, but the interpretation of this is questionable. The reaction remains strong when up to five joints are amputated and then it declines. When only one or more of the last three joints remain, a reversed reaction occurs, similar to that shown by desiccated individuals.

7. This suggests that there are two kinds of humidity receptors, the first kind being mainly located distally on the antennae and some of the second kind being either on the basal joint or not on the antennae at all.

8. Attempts to identify humidity receptors were not successful.

In conclusion, the writer would like to express his appreciation of the help and advice rendered by Dr D. L. Gunn and the encouragement given by Prof. H. Munro Fox, F.R.S., and Prof. Lancelot Hogben, F.R.S., during the course of the work.

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ON THE MEASUREMENT AND MODIFICATION OF THE OLFACTORY RESPONSES OF BLOWFLIES

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(With Four Text-figures)

Froggat suggested that the native primary blowflies in Australia began to attack living sheep because of a change of habit which resulted in their becoming attracted to their wool for oviposition or larviposition. They were supposed to have acquired this habit during great droughts when the carcasses of sheep formed a large part of the carrion on which they normally oviposited. During these times the flies deposited their eggs, or living larvae, among the wool of the dead sheep. Then 'the smell of the dead wool taught them that the allied damp or soiled wool (of living sheep) was of a similar character', and consequently they became attracted to the latter for purposes of oviposition or larviposition (Tillyard & Seddon, 1933). Although the evidence seems to be overwhelmingly in favour of the belief that in Australia serious blowfly attack on sheep followed the spread of the introduced species of fly, *Lucilia cuprina* and *L. sericata*, Froggat's suggestion was interesting, and it seemed worth while to investigate experimentally the degree to which the responses of blowflies to odours could be modified by their past experience during larval or adult life. Thorpe & Jones (1937), Thorpe (1938–9) and others have, of course, already shown that such changes of behaviour may occur in insects.

As they stand these experiments are unsatisfactory chiefly for the following reasons. First, in several cases the flies began to die off before the experiments were over. The cause of this mortality was apparently not old age, because the average longevity of flies kept in captivity under the same conditions was found to be over 6 weeks for *Lucilia* and over 4 weeks for *Calliphora* (cf. Mackerras, 1933), whereas in one case the experimental *Lucilia* practically all died between the thirteenth and fifteenth days of life (Table 5), and in two experiments almost all the *Calliphora* were dead by the tenth day of life (Tables 3, 4). This suggests that the longevity of the flies was less than usual because of the experimental conditions to which they were subjected. Secondly, the example of 'conditioning' mentioned below needs further investigation. Finally, in the experiments described in § I, the *minimum* change in menthol concentration required to cause a change of response, at different intensities of both light and odour, has not been measured. This could be ascertained by performing more tests at intermediate concentrations of menthol vapour (Fig. 1*a*). The whole purpose of these experiments would, however, have probably been more effectively achieved if, instead of measuring the percentage response, the technique had been used of measuring the different intensities of odour and light which balanced each other, leaving 50% of the flies in each arm of the Y. But as it is likely that circumstances will prevent a return to the subject for the time being, it seems useful to publish the results as they are.

I. THE CONTROL AND MEASUREMENT OF THE OLFACTORY STIMULUS

The responses of the blowflies were tested in an olfactometer which was essentially similar to that used by Thorpe & Jones (1937) and Thorpe (1938), except that the Y-tube was 2 in. in diameter to accommodate the larger insects used here (see Crombie, 1941). The chief defect of this apparatus lies in the difficulty, common to many investigations of the sense of smell (cf. Dethier, 1937; Elsberg, 1937), of measuring exactly the intensity of the olfactory stimulus. An attempt was made to overcome this.

Two species of blowflies were used, viz. *Calliphora erythrocephala* and *Lucilia sericata*. The experimental insects belonging to each species were respectively descended from a single pair of original parents. The flies used in the experiments described in the first section of this paper were one day old. They had been fed from emergence on a diet of crystalline sucrose, meat and water (all renewed daily), and then starved for 4 hr. before being tested in the olfactometer. In all the experiments described in both sections of the paper the adults were, when not being tested, kept in a constant-temperature room, open to the daylight, at 23° C. and 70% R.H. All the olfactometer tests were performed in a blacked-out constant-temperature room at 23° C. and 70% R.H. These insects gave clear-cut responses in the olfactometer. With *Lucilia*, for instance, $151 = 82\%$ (S.E. $= \pm 3{\cdot}7\%$) out of 185 insects went to the arm of the olfactometer containing a bait of high meat. The actual number of insects tested was forty, and each individual was put through the olfactometer about four times. The symmetry of the Y-tube was examined by placing the lamp on the axis of the stem and testing the responses of flies with no 'bait'. It was found that approximately 50% of the flies went into each arm whether the air currents in them were equal or one was ten times as strong as the other, which showed both that there was no bias in the tube affecting the flies' behaviour and that the latter did not respond to wind currents (Hoskins & Craig, 1934). The nature of the air flow in the Y-tube was investigated as follows. To one arm of the Y was attached a tube which led from two flasks, on containing hydrochloric acid and the other ammonia. The white vapour of ammonium chloride then entered the arm of the Y-tube. At the junction of the arms with the stem of the Y there was some turbulence, but in spite of this the streams from each arm remained clearly distinct all the way down to where the air was drawn out. The flies would therefore have the whole length of the stem in which to make their choice. In fact what they did if there was no 'bait' present was to pursue a zigzag course up the stem towards the light, while if there were a repellent or attractant odour coming down one arm they soon made their choice of sides and continued along it until the junction, when they entered the arm on the chosen side.

The strength of the olfactory stimulus, which in this case was provided by menthol vapour, was controlled as follows. Part of the air which entered the 'bait' arm of the olfactometer was passed through tubes containing solid menthol so that it was saturated with the vapour of this substance. This menthol-saturated air was then diluted to any desired degree by introducing a stream of pure air which, with the menthol stream, was passed through mixing tubes before entering the 'bait' arm (cf. Wirth, 1928). The strength of the olfactory stimulus may thus be expressed in terms of the proportion of menthol-saturated air in the air entering the 'bait' arm. The volumes of these two streams of air entering the 'bait' arm were measured by means of flow-meters. Each of the latter had been calibrated by attaching an aspirator to it and measuring the time for 500 c.c. to run out. The pressure difference in the flow-meter was adjusted to each point in turn by a stop-screw on one of the rubber leads, and by the same means the meniscus of the liquid in the U-tube capillary was kept at the point concerned as the water ran out of the aspirator. The flow-meter readings were plotted against the rate of flow of the air, expressed in c.c. per sec., and a curve drawn from which the rate of flow of air corresponding to any reading on the flow-meter could be read. Thus the number of c.c. of air entering the 'bait' arm per second could be determined directly from the flow-meter readings, and the volume of menthol-saturated air expressed as a percentage of the total volume of air entering this arm per second. The rates of air-flow in each of the two arms were also equalized in the usual way by means of flow-meters.

The intensity of the olfactory stimulus may also be expressed in terms of the number of molecules of menthol vapour per c.c. This may be determined as follows from the vapour pressure of solid menthol at the experimental temperature, assuming the atmospheric pressure to be 760 mm. of mercury. The vapour pressure of menthol at 23° C. proves to be 1·04 ± 0·05 mm. of mercury. At this temperature, therefore, the number of molecules per c.c. is

$$\frac{6{\cdot}062 \times 10^{23}}{22{,}414} \times \frac{1{\cdot}04}{760} \times \frac{273}{296} = 3{\cdot}4127 \times 10^{16}.$$

This is a measure of the absolute intensity of the olfactory stimulus, but when only the relative intensity is needed it is more convenient to express it as the proportion of menthol-saturated air in the air entering the 'bait' arm.

The following experiment was performed to test this method of measuring and controlling the intensity of the olfactory stimulus. As described later, when the light was as usual in line with the axis of the stem, about 75% of the flies were repelled by the vapour of menthol coming down the 'bait' arm (vide infra, Table 2). In this experiment, the attracting lamp, instead of being placed on the axis of the stem, was placed on a line making an angle of 45° with it, so that one arm was nearer the lamp than the other. The latter will be called the 'illuminated' arm. The adults of both *Lucilia* and *Calliphora* are of course strongly photopositive, and with the lamp so placed about 90% of the flies go to the 'illuminated' arm when there is no menthol vapour present. By introducing menthol vapour into the 'illuminated' arm, therefore, the attraction to the light may be balanced against repulsion by the menthol vapour at different strengths of both light and odour. It should be noted that the ranges of intensity over which the change in response with change in stimulus are measured by this method will be well above the thresholds of response to both stimuli. The lamp was operated by a good, well-charged accumulator with a variable resistance in series. The strength of the current was measured with an ammeter, which was calibrated to give the values of the brightness of the lamp in metre-candles corresponding to each amperage. This calibration was performed by means of a photoelectric cell, itself calibrated with a standard lamp. The accurate measurement of absolute brightness is it seems a matter of some difficulty, and while this method cannot give accurate values for this variable the values of the relative brightness of the lamp with currents of different strengths will be reasonably accurate. Light and odour were now presented to the flies as opposing stimuli in the following way. The lamp was kept at a fixed distance from the junction of the two arms of the olfactometer, and for each series of observations its brightness was kept constant while the strength of the odour was increased by manipulating the flow-meters. The strength of the light was expressed in terms of the intensity of illumination (metre-candles) at the junction of the arms, which was calculated from the brightness of the lamp by means of the inverse square law. The strength of the odour was expressed, as stated above, in terms of the percentage of menthol-saturated air in the air introduced into the 'bait' arm.

Table 1. *Responses of* Lucilia *to antagonistic stimuli of light and the odour of menthol of different intensities (sixty flies used in each test)*

Intensity of light at junction of arms m.c.	% menthol concentration	No. in menthol arm	No. in 'blank' arm
0·095	0	203	18 = 8%
	10	93	47 = 34%
	25	97	63 = 39%
	50	82	123 = 60%
	75	31	100 = 76%
	100	31	119 = 79%
0·64	0	151	18 = 9%
	10	146	58 = 28%
	25	125	71 = 36%
	50	88	112 = 56%
	75	52	141 = 73%
	100	41	116 = 74%
2·58	0	173	24 = 9%
	10	158	47 = 23%
	25	127	56 = 31%
	50	104	84 = 45%
	75	71	126 = 65%
	100	49	130 = 73%
9·15	0	147	14 = 9%
	10	203	22 = 10%
	25	174	52 = 23%
	50	109	54 = 33%
	75	123	145 = 52%
	100	46	101 = 69%

The experimental procedure adopted was to adjust the suction pump, which drew air from the stem of the Y, to a fixed point so that the volume of air entering each arm remained constant at 5 c.c. per sec. The amount of menthol vapour in the 'bait' arm was then varied by varying the relative amounts of menthol-saturated and pure air entering it, the sum of the menthol-saturated and pure air always remaining constant at 5 c.c. per sec. Four different intensities of illumination were used, at each of which observations were made with menthol of five different strengths and with no menthol. Sixty insects were used in each test and these were put through the olfactometer between two and four times. The results are shown in Fig. 1 and Table 1. The standard error of the proportion of flies entering the 'blank' arm never exceeded 4%. There was no difference between the responses of samples of flies taken from the 'menthol' and 'blank' arms, showing

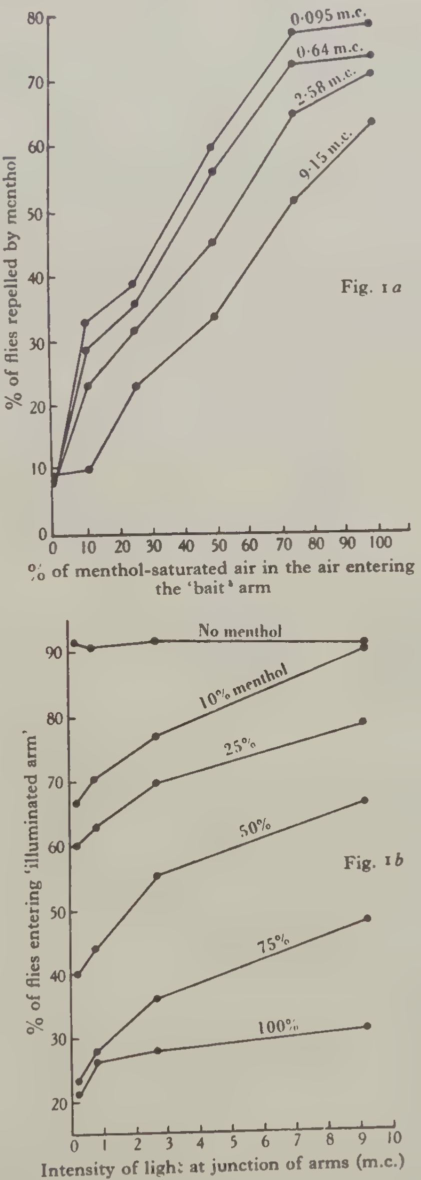

Fig. 1. Responses of *Lucilia* adults to antagonistic stimuli of light and the odour of menthol of different strengths (Table 1). (*a*) Variation in response with constant intensity of illumination but changing concentration of menthol. (*b*) Variation in response with constant concentration of menthol but changing intensity of illumination.

that the population was homogeneous in its responses to these sensations (vide infra).

No theoretical deductions seem to be easily drawn from these results. The chief conclusions will therefore be baldly stated as follows. Fig. 1*a* will be considered first. (*a*) With constant illumination the relationship between the proportion of flies repelled by the odour of menthol and the concentration of this substance seems (within the limits of the experimental error, which is fairly large), to be rectilinear between 10 and 75 % menthol for the first three intensities of illumination, and over the whole range for the highest illumination. (*b*) The flattening of the curves which tends to occur at high concentrations of menthol seems to be due to the fact that the limit of accuracy of the olfactometer has been reached. The flies made an error of choice of about 25 % in their responses to this odour (cf. Table 2). An error of similar magnitude was made in responding to the odour of meat (vide supra). (*c*) Likewise, the flies made an error of choice of about 10 % in their responses to light. Even when there was no menthol vapour in the 'illuminated' arm never more than about 90 % of them succeeded in getting into it. (*d*) The lowest illumination used is above the threshold of response to light, and higher intensities of illumination produced no greater response than lower intensities when there is no menthol vapour present (cf. Brown & Hall, 1936). (*e*) Between 10 and 75 % menthol concentrations all the lines seem to be parallel within the limits of the experimental error (S.E. × 2). (*f*) It appears from Fig. 1*b* that with constant concentration of menthol vapour the proportion of flies going towards the light is not linearly related to the intensity of illumination. The range of intensities of illumination tested is, however, too small for the exact relationship to be ascertained here. It may be noted that when the light was placed on the axis of the stem of the Y-tube (Table 2), i.e. when there was no attraction by light towards either arm, the proportion of flies entering the 'bait' arm (with 2 % menthol) was 24·5 %. The five bottom curves in Fig. 1*b* would probably tend to approach this point at the left-hand end. (*g*) These results give some indication of the degree of accuracy to which the olfactory stimulus has been controlled. The intensity of illumination can be relatively accurately controlled, and the control of the intensity of the olfactory stimulus is, like light, evidently of an order of accuracy at least equal to that of the apparatus in measuring the response. In different regions of intensity of light and odour, changes of 10, 15 and 25 % produced statistically significant changes in response (see Table 1). Furthermore, the behaviour of the flies in the apparatus is what it would be expected to be: in each case a greater intensity of stimulus tended to produce a greater response, and the relationship between stimulus and response is more or less regular and orderly. The regular way in which the response is balanced between the two opposing stimuli at different intensities of the latter recalls the observations on responses to light and gravity of Crozier & Pincus (1927) with the rat and of Yagi (1928) with *Dixippus morosus*. No theoretical conclusions seem to be easily deducible from this behaviour, however, although it perhaps points to the operation of some exact physiological or psychological factor. It should be noted that the method of orientation to the two stimuli would be different: that to the odour of menthol is probably a combination of klino-taxis and tropo-taxis, and that to light telo-taxis (Fraenkel & Gunn, 1940).

Table 2. *The responses to menthol of insects reared in artificial medium and tested on emergence (controls: group A)*

Sample	No. in menthol arm	No. in 'blank' arm	No. insects used
Calliphora			
Whole	199	625 = 76 %	110
Menthol	59	138 = 70 %	40
Blank	65	162 = 71 %	70
Lucilia			
Whole	89	243 = 76 %	70
Menthol	64	150 = 70 %	30

II. THE MODIFICATION OF THE OLFACTORY RESPONSE BY PAST EXPERIENCE

The insects were reared in a sterile artificial medium (Lennox, 1939) which had been autoclaved. Before being introduced into it the eggs were sterilized by washing with 0·5 % Agral I solution and treating with 0·1 % mercuric chloride for 15 min. (Hobson, 1932). Five groups of flies of each species were reared: (A) Controls were reared on the artificial medium and their response to menthol tested on emergence (Table 2). (B) The second group was reared from hatching on artificial medium containing 0·2 % by weight of menthol. When feeding had finished the larvae were washed in distilled water and removed to clean sawdust and the response of the adults to menthol tested on emergence (Table 3, Fig. 2). (C) The third group consisted of *Calliphora* only and was reared like the controls on the artificial medium, but when they had finished feeding the larvae were transferred to clean sawdust through which air containing 10 % of menthol-saturated air was passed until pupation occurred. The pupae were then washed in distilled water and the response of the adults to menthol tested on emergence (Table 4, Fig. 3). (D) The fourth group was reared like the controls on artificial medium but the adults were allowed to emerge into an atmosphere containing 2 % of menthol-saturated air. Their response to menthol was tested after 3 days in this atmosphere in the case of *Calliphora*, and after 11 days in the case of *Lucilia* (Table 5, Fig. 4). (E) The fifth group consisted of *Calliphora* only and was treated like the fourth except that the adults did not emerge into the atmosphere of menthol but were placed in it when they were several days old. Adult flies belonging to all groups were starved for 4 hr. before being tested in the olfactometer, but otherwise were fed as before on a diet of crystalline sucrose, meat and water. The latter were renewed daily. Freshly emerged flies belonging to the first three groups were fed, before being starved for the standard 4 hr. While the tests were being carried out the adults were in all cases kept in a menthol-free atmosphere and fed on the diet mentioned above. There is thus a series of flies which were exposed to a specific chemical sensation during progressively later periods of their life and, as described below, their behaviour towards it was modified according to the stage in which they first experienced this sensation. The response of each group as a whole was tested first. In all cases the flow-meters were adjusted so that the air entering the 'bait' arm contained 2 % of menthol-saturated air. The lamp was,, of course, placed on the axis of the stem of the Y so that the flies were

Table 3. *The responses to menthol of adults reared from hatching until the end of larval feeding in sterile artificial medium impregnated with this substance (group B)*

Days after emergence	Sample	No. in menthol arm	No. in 'blank' arm	No. insects used
Calliphora				
0	Whole	142	125 = 47 %	87
	Menthol	68	52 = 43 %*	36
	Blank	34	78 = 70 %*	40
2	Whole	109	88 = 45 %	50
6	Whole	73	117 = 62 %	30
10	Whole	18	39 = 68 %†	12
Lucilia				
0	Whole	97	87 = 47 %	55
2	Menthol	80	80 = 50 %	30
	Blank	18	93 = 83 %‡	25
6	Menthol	42	112 = 73 %	25

* S.E. = 4·6 %. † S.E. = 6·6 %. ‡ S.E. = 4·7 %.

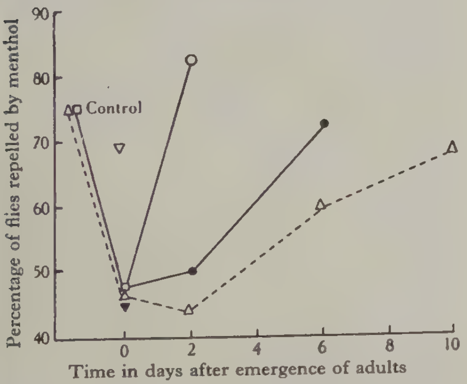

Fig. 2. Responses to the odour of menthol of adults reared from hatching to the end of larval feeding in sterile medium impregnated with this substance (group B, Table 3). *Calliphora*: △ whole population (A+B); ▼ menthol sample (A); ▽ 'blank' sample (B). *Lucilia*: □ whole population (A+B); ● menthol sample (A); ○ 'blank' sample (B).

Table 4. *The responses to menthol of* Calliphora *adults exposed to the odour of menthol as post-feeding larvae (group C)*

Days after emergence	Sample	No. in menthol arm	No. in 'blank' arm	No. insects used
	Conditioned at 23° C. for 8 days			
0	Whole	103	106 = 51 %	100
2	Whole	79	100 = 56 %	100
	Menthol	81	52 = 39 %*	58
	Blank	54	100 = 65 %	42
4	Menthol	127	106 = 46 %	52
6	Menthol	100	142 = 59 %	50
	Blank	68	148 = 69 %	40
10	Menthol	85	200 = 70 %	40
	Blank	38	118 = 76 %	16
	Conditioned at 0° C. for 30 days			
0	Whole	167	173 = 51 %	85
2	Whole	120	175 = 59 %	75
3	Whole	97	242 = 71 %	73
4	Whole	100	246 = 71 %	73
6	Whole	51	217 = 81 %	52

* S.E. = 4·3 %.

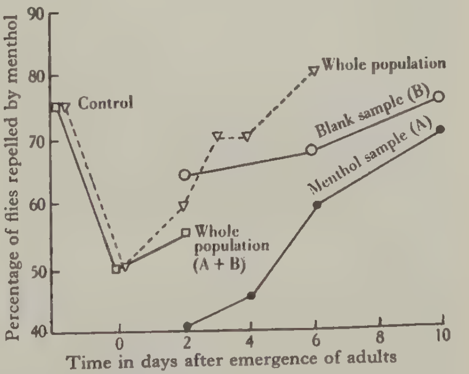

Fig. 3. Responses to the odour of menthol of *Calliphora* adults exposed to this odour as post-feeding larvae (group C, Table 4).

attracted towards the arms against the air stream. It was found that the best results were obtained when the lamp was very dim. The intensity of illumination was 0·095 m.c. at the junction of the arms of the Y. With a bright lamp the flies tended to become excited and attempt to fly, while when it was dim they crawled quietly along the Y-tube and responded well. When the whole group had been tested the insects going to either arm of the olfactometer were removed from the traps concerned and kept to form the 'menthol' and 'blank' samples. These were then tested separately every 2 or 4 days. The actual number of insects used in each test is shown in the last column of each table. The same insects were put through the olfactometer between about two and ten times in different experiments. At the end of each experiment all the flies were sexed, as were any of them that succumbed before it was finished. The standard error of the proportion of flies entering the 'blank' arm never exceeded 4% except where it is indicated to the contrary in the tables.

Table 5. *The responses to menthol of adults which have spent several days from emergence in a 'conditioner' in an atmosphere of this odour (group D)*

Days after removal from 'conditioner'	Sample	No. in menthol arm	No. in 'blank' arm	No. insects used
	Calliphora (3 days in 'conditioner')			
0	Whole	154	183 = 54 %	90
	Menthol	77	103 = 57 %	35
	Blank	60	160 = 73 %	55
2	Menthol	72	139 = 66 %	30
6	Menthol	42	100 = 70 %*	30
	Lucilia (11 days in 'conditioner')			
0	Whole	104	145 = 58 %	60
	Menthol	91	99 = 52 %	25
	Blank	71	150 = 68 %	33
2	Menthol	53	93 = 64 %	25
	Blank	50	123 = 72 %	30
4	Menthol	42	73 = 64 %*	10

* S.E. = 4·6 %.

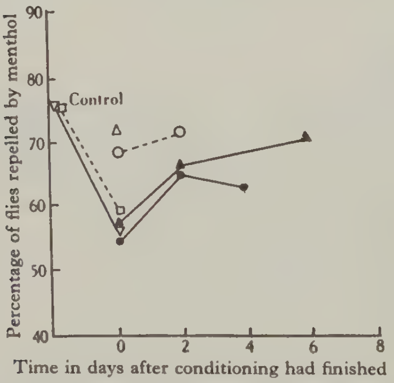

Fig. 4. Responses to the odour of menthol of adults exposed to this odour immediately on emergence (group D, Table 5). *Calliphora*: ▽ whole population (A+B); ▲ menthol sample (A); △ 'blank' sample (B). *Lucilia*: ☐ whole population (A+B); ● menthol sample (A); ○ 'blank' sample (B).

The controls (A) of both species were strongly repelled by menthol (Table 2). The responses of flies of both species belonging to groups B, C and D are shown in Tables 3–5 and Figs. 2–4, respectively. In each case the adults achieved a tolerance of the odour of menthol which gradually disappeared after a few days in a menthol-free atmosphere. By 'tolerance' is meant, of course, that in the olfactometer 50 % of the insects went to each arm, i.e. the menthol did not affect the 'chance distribution', so that it may be concluded that the flies are not responding to it. The response of the flies to menthol was thus modified when they first experienced this normally repellent sensation in the larval stage or immediately upon emergence. If, however, as in group E, they were first exposed to the odour of menthol a week after emergence as adults, their behaviour towards this sensation was not modified. Thus ninety *Calliphora* adults, which had been exposed to the odour of menthol for 2 weeks beginning when they were a week old, were still repelled 230 = 73·5 % (S.E. = ± 2·83 %) out of 313 times by this odour, and thirty *Lucilia* adults which had been similarly treated were repelled 107 = 75·4 % (S.E. = ± 4·19 %) out of 142 times by the odour of menthol. It may therefore be concluded that *immediately after emergence* the adults are in a plastic state in which they are more easily influenced by the external situation than when they are older.

There is a possible objection to the conclusion that the change in response to the odour of menthol which occurred in groups B and C was due to the memory of a larval experience, for if the contents of the Malpighian tubes reached the ecdysial fluid, either with the faeces via the rectum as in the silkworm (Esperon, 1937) or via the perivisceral fluid as in *Acidia* (Keilin, 1921), and became spread out over the inner surface of the puparium, then the adult might experience the odour of this substance just before its emergence from the puparium. Eastham (1925), however, found that the contents of the Malpighian tubes of *Drosophila funebris* were passed to the gut about the sixth day of pupal life, and only expelled from the body on the emergence of the adult. The fate of the contents of the Malpighian tubes at metamorphosis of *Lucilia sericata* and *Calliphora erythrocephala* was investigated by the following experiment, which was devised by Thorpe (1939). The larvae were fed on minced meat to which sufficient carmine had been added to give a bright red colour. After they had ceased feeding the larvae were washed in distilled water and placed in clean sawdust to pupate. A few days before emergence some of the pupae were dissected out of the puparia and both examined for carmine. None was found either between the pupa and puparium, nor, after adults had emerged, inside the pupa case itself. These observations were made with carmine because it is easy to see, but it is probable that the fate of menthol would be the same. It may therefore be concluded that the above objection does not hold and that an alteration has been effected in the larval nervous system by their experience in this stage and has survived metamorphosis to influence the behaviour of the adults (cf. Thorpe & Jones, 1937; Thorpe, 1938–9; Cushing, 1941).

When the 'menthol' and 'blank' samples from groups B, C, D are compared it is seen that there is a marked difference in their responses to the odour of menthol, and that in fact the behaviour of the latter sample has scarcely been modified at all. Thus when a population of flies appears in the olfactometer to be 'tolerant' to an odour each individual fly is not necessarily indifferent to it, but some may be repelled and some attracted. A statistical method such as this obscures this result and may give a totally wrong impression of the situation. No such difference was observed in the two samples of the controls (A) (Table 2), both being equally repelled. The treatment with menthol has therefore brought about a division of the population into two sections differing in their responses to this odour, and it is conceivable that such behaviour may lead to genetic isolation and thus to the formation of 'biological races' (Thorpe, 1930). It should be pointed out, however, that the modification of response to the odour of menthol of flies which had been exposed to this odour as larvae had, in almost all cases, disappeared by the end of the pre-oviposition period (approximately 6 days for *Lucilia* and 10 days for *Calliphora*). After this it would therefore cease to be a factor of isolation. But it remains possible that the effects of larval experience of less repellent odours may not disappear so quickly. It would be interesting to know what causes the difference in behaviour of blowflies belonging to the two samples. There are cases in which variations in the behaviour of members of the same species are known to have a genetic origin (e.g. McEwen, 1918, 1925; Brown & Hall, 1936), although there is no evidence that this is so here.

The change in behaviour observed in Tables 3–5 may probably be described as 'habituation'. Thorpe (1943*a*) defined habituation as 'an activity of the central nervous system whereby innate responses to mild shock and warning stimuli wane as the stimuli continue for a long period without unfavourable result'. The insects became accustomed to the odour of menthol and ceased to respond to it (Humphrey, 1933). In one case, however, when *Calliphora* had been exposed to menthol as post-feeding larvae, the flies of the 'menthol sample' seem actually to have been attracted by the odour of this substance (Table 4). These flies thus seem to have become 'conditioned', their behaviour changing from negative to positive (see Crombie, 1941), although it must be admitted that the results fall under the shadow of a statistical doubt. Thorpe (1938–9) suggested in explaining his results with *Nemeritis* and *Drosophila* that the modification of behaviour is not as in Pavlov's 'conditioned reflex' an association between a definite stimulus and a definite reaction, but rather between one constituent of the environment (e.g. the odour of menthol) and optimum conditions of life in general. The post-feeding larvae perform no functions such as feeding, oviposition, etc., but merely walk about until they pupate. The odour of menthol could therefore not have become associated with any specific function, and thus appears to have been associated with a favourable environment as a whole. Furthermore, during the period of their exposure to menthol this odour could have had no particular meaning for the animals, but must have been merely one of many other sensations which they experienced. It was only when the odour was experienced again out of its context, so to speak, in the olfactometer, that it acquired significance and became a signal for that pattern of comfortable sensations of which it was a part when first encountered. This sensation acquired significance (cf. Russell, 1938, 1941), then, only after it had been learnt; when it was learnt it had no specific meaning. This is perhaps an example of 'latent learning', or 'learning without patent reward'. According to Thorpe (1943*a*), 'Latent learning experiments seem to involve the preliminary learning of a locality or environment as a whole together with the ability subsequently to select certain parts of that whole for use as guides or signals for the whole or for some other part thereof or in connexion with a new motivation' (see also Thorpe, 1943*b*).

Post-feeding larvae (C) may be prevented from pupating by keeping them at 0° C. Under these conditions they were exposed to the odour of menthol for 30 days, but except that the habituation seemed to disappear more quickly the result was identical with that obtained after an exposure of 8 days at 23° C. (Table 4, Fig. 3).

The 'habituated' flies of both species were tolerant of the odour of menthol when the air entering the 'bait' arm of the olfactometer contained 2% of menthol-saturated air. When, however, the diluting flow-meter was shut off so that the air entering the 'bait' arm was 100% menthol-saturated, then the 'habituated' flies were just as strongly repelled as the controls, i.e. about 75% of them went to the blank arm. The strength of the odour (above 2%) entering the 'bait' arm made no difference to the proportion of controls repelled. When the menthol was as strong as this it probably acted as an irritant, in which case one would expect the olfactory responses to be obscured or upset. No difference was observed between the responses of the two sexes in any of the experiments.

Thus although the evidence is against it being the cause of economic damage to sheep in Australia, the responses of blowflies may be modified by their past experience as larvae or as young adults, and Froggatt's theory, although improbable, is possible.

III. SUMMARY

The accuracy of the control of the olfactory stimulus was tested by balancing olfactory and visual stimuli of different intensity. This demonstrated that a reasonably accurate control of the intensity of the olfactory stimulus was achieved, and also disclosed some facts about the quantitative relationship of stimulus to response.

These flies are normally repelled by menthol but their response to the odour of this substance was modified when they first experienced it in the larval stage or immediately upon emergence. The memory of an experience in the larval stage thus survives metamorphosis and affects adult behaviour. Most of the flies then became 'habituated' to the odour, but in one case they appear to have become 'conditioned'. The latter may be a case of 'latent learning'. There was no modification of the response of adults which first experienced the odour when they were several days old. The populations of habituated or conditioned flies were not homogeneous. Different samples of populations which seemed at first to be indifferent to the odour of menthol often proved to respond differently, some being repelled by it, others indifferent or even attracted to this odour.

I should like to thank Dr A. D. Imms, F.R.S., for his interest in these experiments, Dr F. S. Dainton for kindly measuring the solid vapour pressure of menthol, and especially Dr W. H. Thorpe for helping me to clarify my ideas on the subject. This work was done while holding a research scholarship from the University of Melbourne.

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THE BIOLOGICAL ACTION OF IONS AND THE CONCENTRATION OF IONS AT SURFACES

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(With One Text-figure)

1. INTRODUCTION

In this paper a new approach is made to the problem of the biological action of ions, particularly to the large class of antagonistic or balanced actions such as the antagonistic action of sodium and calcium which is important in determining cell permeability, in the behaviour of the heart, in the survival of marine organisms in artificial media, for the optimum development of plants, etc. In these balanced actions it is common for the action of one calcium ion to be balanced by the action of 50–100 sodium ions.† It is very difficult to see what kind of physico-chemical mechanism can enable one calcium ion to offset the action of 25 or more sodium ions. The modern theory of strong electrolytes in homogeneous aqueous solution has completely failed to provide any clue for the solution of this problem. However, the situation would be much simplified if it could be shown that at the actual site of action of the ions the ionic ratio is not of the order of 50:1, as in the environmental fluid, but more nearly approaches 1:1.

There is much evidence to suggest that the actual sites upon which such ions as calcium exert their action are surfaces, particularly the cell surface and the surfaces of protein molecules. These surfaces are usually charged. It has long been implicit in the theory of colloid chemistry that the concentration of ions in the immediate vicinity of a charged surface is substantially different from the concentrations in the media bathing those surfaces, and in several papers (Danielli, 1937, 1941; Hartley & Roe, 1940) it has been clearly demonstrated that in particular the hydrogen-ion concentration at a surface may be up to 10 or even 100 times different from that in the surrounding media. In these papers methods have been developed for calculating the concentrations of ions in the interior of the electrical double layer of a surface. By applying these methods to biological systems we can therefore calculate the actual concentrations of ions at the surfaces upon which the ions act. Wilbrandt (1939) has previously shown that consideration of the Donnan equilibrium between the cell surface and the environmental fluid leads to significant relationships dealing with the relative action of sodium and calcium upon the permeability of the red cell to water and to potassium.‡

2. THE CALCULATION OF ION CONCENTRATIONS AT SURFACES

There are at present two methods of calculating the concentrations of ions at a surface. In the first place (Danielli, 1937, 1941) we may regard the surface as constituting a distinct phase and calculate the distribution of ions between the environmental phase and the surface phase from the Gibbs-Donnan equilibrium. If the various ionic species considered in any one phase all have the same activity coefficient, or if a given ion has the same activity coefficient in both phases, then we obtain

$$(pH_s)_D - pH_b = -\log\frac{[\mathrm{Na}]_s}{[\mathrm{Na}]_b} = -\log\sqrt{\frac{[\mathrm{Ca}]_s}{[\mathrm{Ca}]_b}} = \log\frac{[\mathrm{Cl}]_s}{[\mathrm{Cl}]_b},\ \text{etc.} \quad (1)$$

Here the suffixes s and b denote surface and bulk (environmental) phases respectively and the suffix D denotes a pH or concentration calculated in this way.

The second method of calculating the surface concentration of ions is that of Hartley & Roe (1940), who have shown that the concentrations may be calculated from the electrokinetic potential, ζ, using the equation

$$(pH_s)_E - pH_b = \zeta/60 = 0{\cdot}325u = -\log\frac{[\mathrm{Na}]_s}{[\mathrm{Na}]_b},\ \text{etc.,} \quad (2)$$

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† For the normal functioning of many tissues a sodium-potassium ratio of about 50:1 is required, but it seems probable that the action of potassium is mainly important in determining the resting potential and the internal potassium concentration of the cells, so that its mode of action is considerably different from that which will be proposed for such ions as calcium.

‡ I have previously discussed with Dr Wilbrandt some of the points made in this paper concerning calcium, and greatly regret that the cutting of communications with Switzerland has prevented our pursuing this enquiry together.

for small particles at 25° C. For large particles such as cells the corresponding expression is

$$(pH_s)_E - pH_b = \zeta/60 = 0{\cdot}217u = -\log\frac{[\mathrm{Na}]_s}{[\mathrm{Na}]_b}, \text{ etc.} \quad (3)$$

The suffix E denotes a pH or concentration calculated from (2) or (3). u is the mobility in μ/sec./V./cm.

It has been shown that the distribution of hydrogen ions between the surface of ovalbumin molecules and the bulk phase is the same whichever of these two methods is used (Danielli, 1941). The experimental data required for the first method are the ionic composition of the bulk phase, the net number of positively or negatively charged groups fixed on the surface of the protein molecule, and the total surface area of the protein molecule. The data required by the second method are the ionic composition of the bulk phase and the electrokinetic mobility or potential.

Let us now consider the concentrations of sodium and calcium ions at the surface of the protein molecule. Some of the necessary information is lacking for serum proteins, so we shall make this calculation for ovalbumin. The hydrogen-ion concentration at the surface of ovalbumin molecules has been given elsewhere (Danielli, 1941). Let C_s be the total concentration in electrical equivalents of sodium and calcium ions in the surface of an ovalbumin molecule. Then we have, if $[H^+]_s$ is small,

$$2[\mathrm{Ca}^{++}]_s + [\mathrm{Na}^+]_s = C_s \text{ equivalents.} \quad (4)$$

Also, from the Gibbs-Donnan relationship, we have

$$\frac{[\mathrm{Na}]_s^2}{[\mathrm{Na}]_b^2} = \frac{[\mathrm{Ca}^{++}]_s}{[\mathrm{Ca}^{++}]_b}. \quad (5)$$

Solving these two simultaneous equations we have

$$[\mathrm{Na}^+]_s = \frac{[\mathrm{Na}^+]_b^2}{4[\mathrm{Ca}^{++}]_b}\left\{-1+\sqrt{\left(1+\frac{8[\mathrm{Ca}^{++}]_b}{[\mathrm{Na}]_b^2}C_s\right)}\right\}. \quad (6)$$

All the quantities on the right-hand side of this last equation can be measured experimentally. The method of calculating C_s is given by Danielli (1941). Having obtained $[\mathrm{Na}^+]_s$ from this equation we can most conveniently calculate $[\mathrm{Ca}^{++}]_s$ from equation (5). Table 1 shows results calculated in this way for ovalbumin molecules in an environment 0·133 M in NaCl and 0·004 M in $CaCl_2$. This corresponds to the concentrations used by R. F. Loeb in a systematic study of the binding of calcium by egg albumin (1925). The last column of the table contains the ratio of sodium and calcium ions in the surface. Whereas in the environment this ratio is always 25:1, in the surface the ratio varies from 113:1, when the protein is positively charged at pH 2·8, to 17:1 when the protein is negatively charged at pH 7·5. Thus, on the alkaline side, there is a binding of calcium at the surface of the ovalbumin molecules due to the fixed negative charge of the molecules. From the results in Table 1 we can calculate the amount of calcium which is bound in this way in a protein solution containing a known percentage of protein. R. F. Loeb used a 6·2 % solution of ovalbumin. This contains $6{\cdot}2 \times 6{\cdot}06 \times 10^{23}/4{\cdot}2 \times 10^4$ mol. of ovalbumin per 100 c.c. of solution. The volume of the surface phase of one molecule of ovalbumin in 0·133 M NaCl is given by Danielli (1941) as $1{\cdot}08 \times 10^{-19}$ c.c. Consequently the total volume of surface phase per 100 c.c. of solution is

$$1{\cdot}08 \times 10^{-19} \times 6{\cdot}2 \times 6{\cdot}06 \times 10^{23}/4{\cdot}2 \times 10^4 = 9{\cdot}7 \text{ c.c.}$$

Hence, the protein solution may be regarded as made up of 9·7 % of solution having the composition given in Table 1 for the surface phase and of (100 − 9·7) % of the environmental fluid. Since on the alkaline side of the isoelectric point there is an excess of calcium in the surface, the ratio, $[\mathrm{Ca}^{++}]$ in ovalbumin solution : $[\mathrm{Ca}^{++}]$ in environmental fluid, will be greater than unity, and on the acid side of the isoelectric point, the ratio will be correspondingly less than unity. The crosses on Fig. 1 show the calculated values of this ratio, using the information of Table 1. The open circles show the values of a similar ratio obtained by Loeb (1925) by experimental methods. It is evident that ovalbumin actually binds far more calcium than is indicated by the calculation just made, i.e. the amount of calcium which may be bound by the electrical field of the net electrical charge of the ovalbumin molecule is insufficient to account for the total binding of calcium found experimentally. In other words, part of the calcium bound by ovalbumin is behaving as though it formed an undissociated complex with some group in the ovalbumin surface.

Table 1. *Calculation of the concentration of* $[\mathrm{Na}^+]$ *and* $[\mathrm{Ca}^{++}]$ *in the surface phase of ovalbumin molecules in an environment containing* 0·133 M NaCl + 0·004 M $CaCl_2$

pH_b	C_s	$[\mathrm{Na}^+]_s$	$[\mathrm{Ca}^{++}]_s$	$\frac{[\mathrm{Na}]_s}{[\mathrm{Ca}^{++}]_s}$
2·8	0·034	0·034	0·0003	113
3·2	0·04	0·039	0·00035	111
3·7	0·05	0·049	0·0005	98
4·05	0·065	0·064	0·0009	71
4·45	0·088	0·085	0·0016	53
4·8	0·137	0·133	0·004	25
5·7	0·188	0·171	0·0067	25
6·5	0·233	0·22	0·011	20
7·5	0·265	0·25	0·015	17

It is not immediately clear with which groups of the protein molecule calcium is likely to form unionized compounds. But when the titration curve of ovalbumin (see Cannan, 1938) is compared with the results of Loeb, it is found that the amount of calcium binding increases rapidly as the COOH groups of the protein are converted into COO^-. This suggests that calcium is entering into reaction such as

$$\mathrm{Ca}^{++} + \mathrm{COO}^- \rightleftharpoons \mathrm{Ca}^+\mathrm{COO}. \quad (7)$$

Let us assume that this reaction is the one responsible for the binding of calcium over and above that bound by the electrostatic effect. Then we can write for this reaction

$$\frac{[\mathrm{Ca}^{++}]_s\,[\mathrm{COO}^-]_s}{[\mathrm{Ca}^+\mathrm{COO}]_s}=k, \tag{8}$$

where k is a constant. It should be noted that this constant is not the same as the somewhat similar dissociation constants for calcium proteinates obtained by McLean & Hastings (1935) and Greenberg & Power (1931), who were using bulk and not surface concentrations. If we now evaluate k by using the experimental results of Loeb at one pH, we can then calculate the amount of formation of undissociated calcium compound at other pH values, using the same value of k. If the values thus calculated are in agreement with experiment, considerable support will be afforded to the view that the calcium bound in undissociated form is bound by the reaction (7).

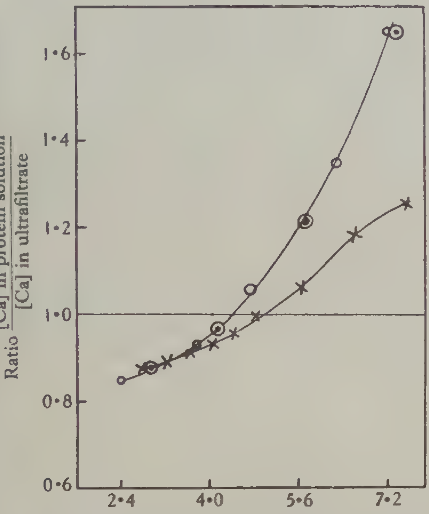


Fig. 1. Calculated and experimental values for the binding of calcium by ovalbumin. ○ experimental values; × calculated for electrostatic binding only; ⊙ calculated for electrostatic binding with association to form unionized calcium-carboxyl complex.

Table 2. *Calculation of the molar concentration of ionic and of undissociated calcium at the surface of ovalbumin molecules in equilibrium with an environment containing* 0·133 M NaCl + 0·004 M $\mathrm{CaCl_2}$

$p\mathrm{H}_b$	$[\mathrm{Ca}^{++}]_s$	$[\mathrm{COO}^-]_s+[\mathrm{Ca}^+\mathrm{COO}]_s$	$[\mathrm{Ca}^+\mathrm{COO}]_s$	$[\mathrm{Na}^+]_s$	$\frac{[\mathrm{Na}]_s}{[\mathrm{Ca}^{++}]_s+[\mathrm{Ca}^+\mathrm{COO}]_s}$
4·1	0·0009	0·38	0·0005	0·064	46
5·7	0·0067	0·69	0·0067	0·171	13
6·5	0·011	0·72	0·011	0·22	10
7·2	0·015	0·76	0·0162	0·24	7·5

To calculate k we must obtain values for $[\mathrm{Ca}^{++}]_s$, $[\mathrm{COO}^-]_s$, and $[\mathrm{Ca}^+\mathrm{COO}]_s$. From the results of Cannan (1938) we can calculate that the ovalbumin molecule has 50 ionized carboxyl groups at pH 7·2. From this information, and from the volume ($1{\cdot}08\times10^{-19}$ c.c.) of the surface phase per molecule of ovalbumin, we can calculate as follows:

$$[\mathrm{COO}^-]_s+[\mathrm{Ca}^+\mathrm{COO}]_s=\frac{1000}{1{\cdot}08\times10^{-19}}\,\frac{50}{6{\cdot}06\times10^{23}}$$

$$=0{\cdot}76\ M \text{ at } p\mathrm{H}\ 7{\cdot}2.$$

Similarly from the analyses of Loeb we can calculate that the total calcium concentration at the surface of the ovalbumin molecule at pH 7·2 is 0·0312, and from calculations such as those of Table 1 we find that $[\mathrm{Ca}^{++}]_s=0{\cdot}015\ M$. The difference between these two figures, i.e. 0·0162, must be the concentration of Ca+COO. Hence

$$k=\frac{0{\cdot}015\times0{\cdot}73}{0{\cdot}0162}=0{\cdot}69.$$

Having obtained the value of k we can now calculate the amount of calcium bound by this mechanism at other pH values. The results of such calculations are given in Table 2. For each pH value it was necessary first to obtain the value of $[\mathrm{Ca}^{++}]_s$, as in Table 1, then the value of $[\mathrm{COO}^-]_s$ from the number of ionised carboxyl groups per molecule at the given pH, and then $[\mathrm{Ca}^+\mathrm{COO}]_s$ can be calculated from equation (8). The final column of Table 2 shows the ratio of the total sodium concentration in the surface to the total calcium concentration in the surface. It will be seen that at pH 7·2 this ratio is reduced to 7·5, compared with a value of 33 in the environment. The closed circles of Fig. 1 show values calculated for the binding of calcium by a 6·2 % ovalbumin solution at various pH, calculated from the data of Table 2 by the same method as used before, but in this case including both the calcium bound in the unionized form and the excess of calcium present as a result of the Donnan equilibrium between the surface and the environment. These points are in good agreement with the experimental values obtained by Loeb. We may therefore conclude that the calcium bound by ovalbumin is bound in two ways: partly as the result of the

Gibbs-Donnan equilibrium between the surface of the protein molecules and the environment, and partly as an apparently undissociated complex between ionized carboxyl groups and calcium.

A somewhat similar finding was obtained by Webb & Danielli (1940) for the binding of calcium by palmitate monolayers. Monolayers of palmitic acid were spread on alkaline solutions containing various concentrations of sodium and calcium. These monolayers were scraped off the surface after they had come into equilibrium with the bulk phase, and their sodium and calcium content determined. It was found that, as the Donnan equilibrium between surface and bulk phase requires, the sodium: calcium ratio in the monolayer is much less than in the bulk phase. But the ratio actually found was smaller than the theoretical ratio by a factor of between 10 and 50: this latter result is again compatible with the formation of an undissociated complex between carboxyl ion and calcium.

It therefore seems probable that many ions form undissociated complexes at interfaces with carboxyl and probably also with phosphate groups. The binding of calcium and also of magnesium by serum protein is probably closely analogous to the binding of calcium by ovalbumin discussed above.

Table 3. *Calculation of the concentrations of ions at cell surfaces from the electrokinetic potential ζ*

1	2 $[\mathrm{Na}^+]_b$	3 $[\mathrm{Ca}^{++}]_b$	4 ζ mV.	5 $[\mathrm{Na}^+]_s$	6 $[\mathrm{Ca}^{++}]_s$	7 Total $[\mathrm{Ca}]_s$	8 $\frac{[\mathrm{Na}^+]_b}{[\mathrm{Ca}^{++}]_b}$	9 $\frac{[\mathrm{Na}^+]_s}{\text{Total }[\mathrm{Ca}]_s}$
Red cell (rat)*	0·145	0·0032	15	0·26	0·01	0·1	45	2·6
Red cell (horse)*	0·145	0·003	13	0·25	0·0087	0·09	45	2·8
Polymorphonuclear leucocyte (horse)*	0·145	0·003	6·6	0·19	0·0051	0·05	45	3·8
Small lymphocyte (horse)*	0·145	0·003	7·8	0·19	0·0051	0·05	45	3·8
Platelet (horse)*	0·145	0·003	6·6	0·19	0·0051	0·05	45	3·8
*B. typhosus**	0·01	—	43	0·052	—	—	—	—
Arbacia egg†	0·5	0·02	30·5	1·6	0·20	2·0	25	0·8
Arbacia sperm‡	0·5	0·02	19	1·05	0·088	0·9	25	1·2
Arbacia sperm‡	0·5	—	22	1·15	—	—	—	—
Cumingia egg†	0·5	0·02	29	1·50	0·18	1·8	25	0·8
Astenas egg†	0·5	0·02	20	1·05	0·088	0·9	25	1·2
Astenas sperm‡	0·5	0·02	23	1·20	0·115	1·2	25	1·0
Astenas sperm‡	0·5	—	27	1·40	—	—	—	—
Echinarachnius egg†	0·5	0·02	20	1·05	0·088	0·9	25	1·2
Echinarachnius sperm‡	0·5	0·02	17	0·95	0·072	0·7	25	1·4
Echinarachnius sperm‡	0·5	—	22	1·15	—	—	—	—
Tubercle bacilli§	0·03	0·0006	8·5	0·042	0·0012	0·01	50	4·2
	0·00023	0·000005	35	0·00088	0·000086	0·0009	50	1·0

* Abramson (1934). † Heilbrunn (1937). ‡ Mudd, Mudd & Keltch (1929). § Freund (1925).

3. THE CONCENTRATIONS OF IONS AT CELL SURFACES

Calculation of the concentrations of ions at cell surfaces can at present be made by three methods: (*a*) from the electrokinetic potential of cells, (*b*) from studies on the exchange of ions between the cell surface and the environment, (*c*) in the case of red blood cells, from analysis of the lipoid constituting the cell membrane.

(*a*) *Calculation from ζ potential.* Calculations are given in Table 3 for various cells using equation (3). The experimental values of ζ are those given by Abramson (1934), Heilbrunn (1937) and Mudd, Mudd & Keltch (1929). The values of the concentration of ionic calcium given in the sixth column of Table 3 are very considerably higher than the ionic calcium concentrations in the environment given in column 3 of the same table. In addition it is necessary to take some account of the unionized calcium present at the surfaces of these cells. The surfaces of most of these cells are believed to consist of a lipoid layer, adsorbed upon which is a layer of protein, Harvey & Danielli (1938), Danielli (1942). From electrokinetic studies it is clear that even in the presence of calcium these surfaces carry a considerable net negative charge which must be due to groups such as carboxyl groups and the phosphate groups of phospholipins. Judging from the results of Webb & Danielli (1940), the concentration of unionized calcium at a lipoid surface may rise to 50 or more times the concentration of ionic calcium when the surface is present in an environment containing a sodium:calcium ratio of the order of 50:1. To get some measure of the order of magnitude of the unionized calcium concentration to be expected at the surfaces of these cells the value for ionic calcium has been multiplied by 10, and rough values for the total calcium at the surfaces of these cells are given in column 7 of Table 3. Of course, this constitutes only a very rough estimate and is not intended to do more than indicate the order of magnitude of the unionized calcium concentration which studies of artificial systems lead us to expect. It is interesting to see that the sodium: calcium ratio for the surface of these cells given in column 9 of Table 3 differs by a comparatively small amount from unity. Thus, our provisional calculations of ion concentrations at the surfaces of cells which are bathed by a physiologically balanced saline suggest that, whereas the sodium: calcium ratio in the saline is of the order of 25:1 or more, the ratio at the surface of the cell, where the balanced action presumably takes place, is approximately unity. This very strongly suggests that the reason for the efficacy of a ratio of the order of 25:1 in physiological saline is that it is this ratio in the bulk phase which is required to give a physico-chemically balanced ratio of the order of 1:1 at the site of action of the ions, i.e. at the cell surface. Some of the results presented in Table 3 for *Echinoderm* sperm were obtained with the sperm in calcium-free media. The value of $[\mathrm{Na}^+]_s$ obtained in this medium is about 0·2 *M* greater than the value obtained for the same sperm in calcium-containing media. This increase is, no doubt, due to the displacement of non-ionic calcium from the surface by sodium ion. It would be of great interest to know the mobilities of cells which had been thoroughly washed in calcium-free solutions, for observations on such cells may make it possible to estimate directly the amount of calcium which is bound at the cell surface in unionized form.

Table 4. *Calculation from the results of Mazia* (1940) *of the area per calcium atom at the surface of various cells*

	Surface area per cell	Mg. Ca per cell	Atoms of Ca per cell	Area per Ca atom
Yeast	3×10^{10} A.^{2}	$2\cdot5 \times 10^{-11}$	$3\cdot8 \times 10^{8}$	80 A.^{2}
Elodea	7×10^{10} A.^{2}	5×10^{-10}	$7\cdot5 \times 10^{9}$	10 A.^{2}
Arbacia egg	$6\cdot9 \times 10^{12}$ A.^{2}	2×10^{-9}	3×10^{10}	230 A^2.

(*b*) *Calculation from exchange of ions*. Mazia (1940) has studied the exchange of ions between the surfaces of cells and the environmental fluid. These ion exchanges are rapid, and can thus be readily distinguished from the exchange of ions between the environment and the interior of a cell, which is a slow process. Using this method, Mazia has shown that in physiological fluids a substantial amount of calcium is present at the surfaces of cells. These results are shown in Table 4 and are recalculated to give the area in A.^{2} occupied by one calcium atom at the cell surface. It will be seen that these areas are quite small. The largest area, that found for *Arbacia* eggs, is about equal to the area occupied by 4 mol. of phospholipin, sterol or triglyceride. The smaller areas per Ca atom recorded for yeast and *Elodea* cells must be regarded with some suspicion, because these cells are surrounded by a rigid framework which probably also contributes to the binding of calcium. But the value for *Arbacia* eggs is probably substantially correct. It is therefore interesting to see that the figure of 1·64 *M*, which corresponds to one calcium atom per 230 A.^{2}, is in reasonable agreement with the estimate of 2·0 *M* given in Table 3 for the total calcium concentration at the surface of *Arbacia* eggs.

Further evidence of the binding of ions at cell surfaces was obtained by Mazia & Mullins (1941), using radioactive copper. It was shown that the concentration of copper at the cell surface could rise by a factor of 10^6 or more above the concentration in the environment. This radioactive copper was so firmly bound that it could hardly be removed by washing with saline, but on the other hand it would exchange almost instantaneously with solutions containing either non-radioactive copper, or divalent gold, thus showing that the radioactive copper had entered into a complex with the cell surface which was in dynamic equilibrium with other ions.

(*c*) *Calculation from lipoid analysis*. Many workers have shown that practically all of the lipoid present in red blood cells must be present in the cell membrane and that even so this membrane can only be about 2 lipoid molecules thick. These lipoid molecules must be oriented with their polar groups towards the lipoid-water interfaces of the membrane (see Danielli, 1942). Consequently, from simple quantitative analysis of red cell lipoid it is possible to obtain a measure of the number of potentially available ionic groups at the cell surface. Dziemian (1939) has made an analysis of red blood lipoids which is suitable for this purpose. Some calculations based on this analysis are given in Table 5. The second column shows the fraction of the surface area of the red cells which will be occupied by the phospholipin if the phospholipin is uniformly distributed throughout the membrane. The calculation is a rough one based upon the proportion of phospholipin to triglyceride and sterol, using the cross-sectional areas of these molecules in monolayers given by Adam (1941). In the third column of the table is given the area per calcium atom assuming that two phospholipin molecules are required to bind one calcium atom. Should only one phospholipin molecule be required then these areas should be halved. In the last column of the table is given the concentration of calcium in the surface phase corresponding to these areas per calcium atom, when the red cells are in a medium of the same ionic strength as blood plasma. The values obtained in the last two columns are, of course, maximal, since it has been assumed that all the phospholipin negative charge is neutralized by calcium, whereas in a physiologically balanced saline some would be balanced by sodium, potassium and magnesium, etc. In addition there will, no doubt, be a certain number of carboxyl groups from the protein molecules adsorbed on the surfaces of the red cells, which will contribute to the number of ions bound by the cell surface. The concentration of calcium obtained in this way for the rat red cell may be compared with the rough estimate for the same cell made in Table 3. The estimate made here is considerably the higher. This may be due to the factor of 10 taken for the ratio unionized : ionized calcium at the cell surface being too low, or to the electrophoretic mobility of the red cells being dominated by a protein layer adsorbed upon the lipoid layer, so that the electrophoretic mobility obtained is characteristic, not of the cell membrane, but of the adsorbed protein in the case of the results presented in Table 3 for red cells.

Table 5. *Calculation of* $[\mathrm{Ca}]_s$ *at the surface of red cells from the results of Dziemian* (1939), *assuming that one calcium atom is bound by every two phospholipin molecules*

	Fraction of surface occupied by phospholipin	Area per Ca atom sq. A.	$[\mathrm{Ca}]_s$ gm. mols per litre
Rabbit	0·51	160	1·25
Rat	0·65	135	1·45
Beef	0·67	133	1·5
Monkey	0·69	130	1·55

In all these calculations it has been assumed that the anions fixed at the cell surface are uniformly distributed over the whole surface. This assumption is unlikely to be more than approximately true and may very well be quite inaccurate. The extent to which it is true can only be determined by further experiments.

4. THE TOXICITY OF IONS

Matthews (1904) suggested that the toxicity of ions would be found to be proportional to their standard electrode potentials. His reason for making this suggestion was that he regarded the resting potential of a cell as being analogous to an electrode potential, in which case it would perhaps be discharged by ions in the same way as ions discharge an electrode potential. We now know that the resting potential of a cell is in many respects quite unlike an electrode potential, so that the toxic action of ions cannot be due to such discharge of the resting potential. However, it has been found by J. R. E. Jones (1940) that Matthews's prediction as to the relationship between standard electrode potential and toxicity is correct. For a number of organisms it was found that, for a given degree of toxic action, the logarithm of the concentration of the toxic metal in the environment is a linear function of the standard electrode potential. This remarkable relationship has not so far been given any theoretical foundation. But we have seen that for cells in their normal environment definite negative charges are present on the cell surface, probably consisting of carboxyl and phosphate groups. It is reasonable to suppose that the action of such ions as calcium is mediated by interaction with these negatively charged groups. It consequently would appear that if a sufficient proportion of these negatively charged groups were inactivated by formation of an unionized complex with a metal not normally present in substantial amount at the cell surface, abnormal behaviour would follow. Let us therefore assume that a given degree of toxicity is produced by conversion of a definite proportion of the anionic groups present at a vital surface of the cell into unionized complexes with the toxic metal. It is not necessary for our present purposes to specify further whether this surface is the surface of the cell or the surface of an enzyme, etc.

The distribution of the ions of any q-valent metal between the surface under consideration and the environment is given by the Gibbs-Donnan equilibrium between the surface and environmental phases:

$$\frac{[M^{q+}]_s}{[M^{q+}]_b}=r^q, \qquad (9)$$

where r is a constant for all metals. In addition, some of the metal will be forming an unionized complex with the anions of the surface; for this we may write the equation

$$\frac{[M^{q+}]_s\,[A^-]_s^{\,q}}{[MA_q]_s}=(K)_M. \qquad (10)$$

If we could evaluate the various values of the equilibrium constant $(K)_m$ for this reaction we should be able to predict the relative toxicity of the different metals. Now if we ignore all specific relationships between the metals and the anions at the cell surface, and all specific characteristics of the metals other than those which enter into the standard electrode potentials of the metals, we can see a close relationship between the process of formation of a metallic ion at the electrode of the same metal, and the process of ionization of the unionized complex formed between the same metal and a given anion. In both cases the process consists essentially of the metal giving up one or more electrons, in the one case to the electrode and in the other to the potential (i.e. incipient) anion. The standard electrode potential may be regarded (see Taylor, 1930) as a measure of the equilibrium constant for the reaction between metallic ions and electrons, whether this reaction takes place at an electrode surface or with a given anion. The equilibrium constant for the reaction in the case of hydrogen can be written in the form

$$(K)_H = \phi e^{+W/\beta RT}, \tag{11}$$

where ϕ and β are constants. Since the electrode potentials E_0 of the various metals are measured relative to the electrode potential of hydrogen, the equilibrium constant for any other metal M will have the form

$$(K)_M = \phi e^{+(W+E_0F)/\beta RT} \tag{12}$$

where F = one Faraday, R = gas constant, T = absolute temperature. Then from equations (9), (10) and (12), we find that the total concentration of unionized metal complex at the surface is

$$[MA_q] = r^q[M^{q+}]_b\,[A^-]_s^q\,\phi^{-1}\,e^{-(W+E_0F)/\beta RT}. \tag{13}$$

This quantity will be constant for different metals if $[MA_q]_s$ is constant, and in this case $[A^-]_s$ = a constant and r = a constant also, at a given pH for ions of any one valency. Rewriting the above expression in logarithmic form we obtain

$$2{\cdot}3q \log r[A^-]_s + 2{\cdot}3 \log [M^{q+}]_b - 2{\cdot}3 \log \phi - \frac{W}{\beta RT} - \frac{E_0F}{\beta RT} = \text{constant},$$

$$\text{or}\quad 2{\cdot}3 \log [M^{q+}]_b = \frac{E_0F}{\beta RT} + \text{constant, for metals of a given valency.} \tag{14}$$

This last equation is the condition for obtaining solutions of ions of equal toxicity for ions of a given valency. If $\log [M^{q+}]_b$ is plotted against E_0, where $[M^{q+}]_b$ are equitoxic concentrations of different metals of the same valency, linear relationships will be obtained. This is the relationship obtained experimentally by Jones. The distance between the parallel straight lines found for the different valencies is given by the term $2{\cdot}3q \log r[A^-]_s$. In the poisoned condition $[A^-]_s$, and therefore r, will probably be small. Consequently, the displacement between the parallel straight lines will be small and to a first approximation all the points may fall on one straight line. The results of Jones are in close accordance with this last prediction.

It appears from this examination that the toxic action of metal ions, in experiments such as those of Jones, is due to the formation of unionized complexes between anions present at an, at present, unidentified surface, and the metal ions.

5. THE UTILIZATION OF IRON BY MARINE PLANTS

It has been shown by Cooper (1935, 1937) that the amount of iron present in the sea as free ions is extremely low, and is approximately $1{\cdot}8 \times 10^{-15}$ g.mol./l. H. W. Harvey (1937) has shown that, if it is assumed that the rate of diffusion of ionic iron into the interior of a plankton organism is the same as the rate of diffusion in sea water, the rate at which iron is taken up by a typical cell will be $1{\cdot}6 \times 10^{-12}$ mg. of iron per day. If the cell is assumed to have a permeability of the same order of magnitude as that of other cells to ions, i.e. 10^{-16} g. atoms/μ^2/sec./mol./l. concentration difference, it can be shown that for the same cell the rate of permeation is of the order of one atom of iron per day. The actual value which is found for the uptake of iron by such organisms is of the order of 8×10^{-8} mg. of iron per day. This is far greater than the value which was calculated by Harvey, assuming the phytoplankton cell membrane to offer no more resistance to diffusion than an equal thickness of water, and enormously greater than the value calculated assuming the cell membrane has a permeability to ions of the same order of magnitude as that of other cells. It is therefore quite apparent that the cells cannot obtain their supply of iron as a result of simple penetration of the dissolved iron of the sea water into the cells by diffusion processes. However, as Cooper has pointed out, there is a considerable amount of iron present in the sea water as comparatively large particles of ferric hydroxide. Judging from the results of Hazel & Ayres (1931), ferric hydroxide in sea-water will be positively charged. In § 3 of this paper we saw that most cells are negatively charged at this pH and that as a result of this negative charge there is an excess of positive ions at the cell surface. For sodium ions this excess can be calculated from the formula

$$\frac{[\mathrm{Na}^+]_s}{[\mathrm{Na}^+]_b} = r, \quad \text{a constant.}$$

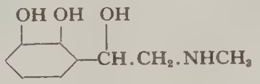
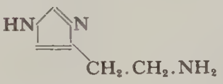
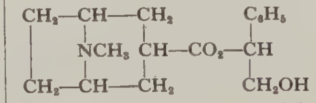
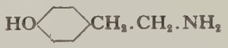
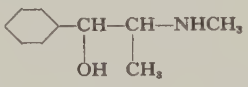
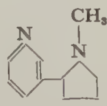
The corresponding formula for n-valent ions is

$$\frac{[M^{n+}]_s}{[M^{n+}]_b} = r^n. \tag{15}$$

As is indicated by the results of Table 3, a common value for the ratio r is 2. Also the colloidal ferric hydroxide particles present in the sea may readily acquire a charge of 10 or 20 units. Inserting this into equation (15) we see that the concentration of iron at the cell surface as colloidal particles may easily rise to be 10^3 to 10^6 or more times greater than the concentration of colloidal iron in the sea water. On this view we should expect to find a high concentration of ferric hydroxide at the surface of phytoplankton: this ferric hydroxide will be available for combination with the anions of the cell surface and may readily be transported into the interior of the cell by any active process such as phagocytosis. These theoretical conclusions are in accordance with the experimental findings of Harvey (1937). He found that ferric hydroxide is readily adsorbed on the surface of diatoms, and that diatoms taken from the open sea have a large amount of iron upon their surfaces, far more than is required for continuous growth. He suggests that this iron is in contact with an interface where its passage into the cell may readily be achieved.

These conclusions may also be true for fresh-water plants, and for land plants.

Table 6. *Calculation of the increase in concentration of a substance at a surface having a specific structure adapted to the substance*

Substance	Formula	Energy of adsorption				
		Electro-static (Donnan)	CH_2 adsorp-tion	Polar group inter-action	Total energy E	$\frac{C_s}{C_b}$
Adrenaline		700	3500	3000	7200	2×10^5
Hystamine		1500	2100	1000	4600	$2{\cdot}5 \times 10^3$
Acetylcholine	$CH_3.CO_2.CH_2.CH_2.\overset{+}{N}(CH_3)_3$	700	4500	?	5200	7×10^3
Choline	$HO.CH_2.CH_2.\overset{+}{N}(CH_3)_3$	700	3500	?	4200	1×10^3
Atropine		700	5000	?	5700	$1{\cdot}5 \times 10^4$
Ergot		700	3500	1000	6200	$2{\cdot}5 \times 10^4$
Ephedrine		700	4200	1000	5900	2×10^4
Nicotine		1500	4200	?	5700	$1{\cdot}5 \times 10^4$

6. THE ADSORPTION OF DRUGS, ETC., AT SPECIFIC SURFACES

Many physiologically active substances produce their effects under physiological conditions in concentrations of 1:10⁶ or less. These substances, such as adrenaline and acetylcholine, must act upon the cell surface, or upon certain enzyme systems. In either case they must act within a surface phase, and it is somewhat difficult to see how the concentration of these molecules may rise to a sufficient value in the surface phase to exert a significant chemical effect. This difficulty may be to some extent surmounted if all the various ways in which a molecule may be adsorbed are able to act simultaneously. The chief mechanisms giving rise to adsorption in biological systems are: (*a*) the partition of ionic substances between surface and bulk phases due to charges fixed on the surface, as considered in earlier sections of this paper; (*b*) the adsorption of hydrocarbon groups at oil-water interfaces; (*c*) adsorption due to the interaction between the polar groups of a molecule and the polar groups of a surface; these interactions have recently been studied by Schulman & Rideal (1937). For a molecule such as adrenaline any one of these modes of adsorption will be insufficient to increase greatly the concentration of adrenaline at a surface. But if all three mechanisms can operate simultaneously, then a very considerable concentration of adrenaline may appear at the adsorbing surface. This adsorbing surface, to produce its effects, will need to have a unique steric arrangement so that the electrical charge on the surface, the oil-water interface, and the particular polar groups of the surface with which interaction is to occur, may all be presented to the adsorbed molecule in just the right position.

In Table 6 results are shown of calculations for a number of physiologically active substances. The third column shows the energy of adsorption in calories due to an electrokinetic potential of 30 mV. This will be of the order of 700 cal. for one positively charged group, 1500 cal. for two charged groups, etc. The fourth column shows the energy of adsorption in calories, due to the hydrocarbon part of the molecule. This is known from the work of Langmuir (see Adam, 1941) to be of the order of 700 cal. per CH_2 group. The fifth column shows the energy of adsorption in calories, due to interaction between polar groups. This has been given a value of 1000 cal. per polar group, which in the opinion of Dr Schulman is probably an under-estimate. This factor includes hydrogen bond formation. In the sixth column is given E, the total of these energies, and in the seventh column the ratio of the concentration of the drug at the adsorbing surface to the concentration in the bulk phase, calculated from the Boltzman equation

$$\frac{[\text{Drug}]_s}{[\text{Drug}]_b}=e^{E/RT}. \qquad (16)$$

Although minimum values were taken for the individual energies of adsorption, the concentration ratios shown in Table 6 show that the concentration in the surface may often be 10^4 times greater than that in the bulk phase, and that higher concentration ratios may quite possibly occur in nature, due to these effects.

This three-factor steric theory of specific adsorption may prove to be of value in connexion with selective dyeing and staining, and in connexion with the specific relations between enzymes, coenzymes, enzyme substrates and enzyme poisons. The work of Quastel & Yates (1936) on the action of dyestuffs on invertase and of Quastel & Tennenbaum (1937) on the inhibition of acetylcholine action by morphine are particularly suggestive in this connexion.

SUMMARY

1. It is pointed out that at the surface of a cell the absolute concentration of ions, and the ratios of the concentrations of the individual ions, are usually different from the corresponding quantities in the environmental fluid. It is suggested that the necessity for a Na:Ca ratio of the order of 50:1 in a physiologically balanced fluid is found because such a ratio in the environmental fluid produces a Na:Ca ratio of the order of unity at the cell surface.

2. The concentrations of calcium and sodium ions at the surface of ovalbumin molecules are calculated. It is shown that calcium is bound (*a*) by electrostatic forces, (*b*) by formation of apparently unionized complexes with carboxyl groups. The complex formation obeys the law of mass action.

3. The ratio, total Na: total Ca at the surface of various cells in physiological media is calculated from the electrokinetic potentials of the cells. The calculated ratio is of the order of unity. In two cases calculation of the maximum possible total Ca at the cell surface by two other independent methods gives values of the same order of magnitude as the calculation from electrokinetic potentials.

4. It is shown that, where the toxic action of ions is due to complex formation with anions present at a surface, the logarithms of the equitoxic concentrations of the metals should be a linear function of the standard electrode potentials of the metals.

5. Calculations show that the concentration of colloidal Fe at the surface of organisms in sea-water should be far greater than the concentration in the environment: it is suggested that marine plants derive their iron from this layer of adsorbed colloidal iron.

6. A mechanism is suggested which provides for intense adsorption of drugs at specific surfaces.

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